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# ON THE OCCURRENCE OF COPULATION INFECTION AMONG CATTLE WITH INFECTIOUS ABORTION.

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THE problem concerning the significance of the bull in the spreading of infectious abortion in cattle keeps pressing on, and additional light on this question is urgently required. Naturally, it is of great interest to the veterinary authorities as well as to the stock-breeding farmers to have the question settled whether or not we have to reckon with a spread of the infection through copulation and, if so, to what extent.

In a previous paper* dealing with this question (1928) I gave an account of the problem at that time, based on various clinical observations in connection with experiments carried out by other investigators and by myself. I pointed out that the original view of infectious abortion in cattle was chiefly a disease of coition—in short, a disease which was spread by the bull—is no longer tenable.

Various conditions discussed in that paper were in conflict with the assumption that the bull could play even a moderately important rôle in the spreading of this infection. A most striking argument against that view was furnished by the experiments reported by several investigators, especially American, in which it had not been possible to infect the cows by direct contact in the act of copulation.† Similar experiments were carried out with

*Den kgl. Veterinær- og Landbohöjskoles Aarsskrift 1928, p. 76. Deutsche tierärztliche Wochenschrift, *36*; 477, 1928.

†Such experiments have been continued in U.S.A., but still without any takes. (See Hadley and Osborn, Journ. Am. Vet. Med. Ass., *81*; 46, 1932.)

goats in the laboratory here, with the same result. It will suffice here to mention that in these goat experiments the infection could not be spread by the male in the act of covering. The male employed in these experiments was infected very strongly just before each copulation by the introduction of a large amount of infectious material into the prepuce—and in this condition it was used to cover 18 goats. But although the goat may be said to be generally rather susceptible to infection with the *Br. abortus*, none of the 18 goats aborted.

In view of these experiments and other conditions mentioned in my first paper, I was, like the American authors, inclined to think it reasonable to consider the bull not an important factor in the *direct* spread of infectious abortion (that is, transmission of the infection through the act of copulation). In my paper, however, I also emphasised that it was desirable that here in Denmark we also might be able to carry out such experiments with bulls, besides the aforementioned goat experiments.

Since then, the establishment of the experimental farm "Tavlebakkegaard" has given the Serum Laboratory sufficiently large grounds and quarters for such experiments as have been going on in the last years. It is these experiments that are reported in the following.

In view of the discovery that about 10 per cent. of reacting bulls (according to American investigations) would produce infected semen, it is obvious that experiments on the transmission of the infection by such bacillary excreters (*e.g.*, suffering from orchitis) will be of considerable interest. Suitable bulls of this kind have proved so difficult to obtain, however, that such experiments as have been carried out in the Serum Laboratory in the last two years have not yet led to any decisive result.

Orchitis and vesiculitis, etc., of this nature are relatively rare phenomena, however, so that the question—in particular the practical point of special interest to the breeders—is: whether a bull which covers a casting cow and thus obtains infectious matter on the penis will transmit this infection to a normal cow in the following copulation. The experiments here presented were designed to contribute to the solution of this question. Still, I wish to point out that they are hardly equivalent quantitatively to the conditions met with in practice.

The experiments are made with three bulls and 15 heifers, besides four control heifers. All the experimental animals were purchased from abortion-free stocks; further, these animals were found to be reaction-free in the blood tests performed just before the experiment.

Immediately before each copulation the bulls were infected in the following manner: At least two pieces of placenta (from two cows), strongly infected with abortus bacilli, were ground in a mortar with suitable addition of sodium chloride solution. Ten c.c.

of this markedly turbid saline-exudate mixture was injected into the preputium in such a manner that none of it could escape unless the prepuce was massaged strongly in the caudal direction. This procedure ensured that the penis was bathed in exudate and therefore strongly infected, but it gave no discomfort whatever to the bulls, which were willing immediately afterwards to cover the heifers. In these experiments, then, the time of the fertilisation of the heifers is coincident with the application of the infectious material.

The covered and hence simultaneously infected animals were stabled under such conditions as would reasonably exclude the possibility of secondary infection (infection later on in pregnancy, for instance, through the feed). As a rule, the heifers were stabled in pairs in the same kind of entirely isolated stable boxes; furthermore, the individual animals of each pair were again separated by a partial partition. Judging from the last experimental series, comprising four heifers and four control heifers (covered by a non-infected bull, and hence non-infected), secondary (accidental) infection may be ruled out in these cases. All the four control heifers that were stabled side by side with the experimental heifers proper gave birth to normal offspring and showed no demonstrable abnormal blood reaction throughout pregnancy.

The following tabulation of the experiments gives in detail the findings in the respective animals.

As shown in Table I, five of the heifers infected by the bull have aborted, while five have calved normally, and five were non-fertilised. Among the five heifers with normal calving, four were free from reaction throughout the gestation, while one (No. 36) showed a slight reaction about the time of calving (two months before calving there was no reaction). As the afterbirth was lost (eaten by the heifer), it is not known whether it was infected.

Of the five non-fertilised heifers, two (Nos. 57 and 191) showed a weak blood reaction several months after the period of heat (and application of infectious material).

In this table it is further seen that the production of antibodies takes place only very slowly after this form of infection; it takes several months before the absorption of infectious material manifests itself in the serological picture. Sometimes it even happens that abortion takes place before any true reaction has been demonstrated.

*In the aborting heifers infected by the bull the incubation period has been strikingly long, averaging 225 days*; the longest was 251 days, the shortest 193 days.

*A priori*, one would hardly have imagined the possibility of such a long incubation period, but these experiments leave no doubt about this fact. In another series of experiments it was found that *the length of the abortion-incubation period (the*

*interval between infection and abortion) is inversely proportional to the stage of pregnancy*—as illustrated in Table II. All the heifers employed in this experiment were infected by the mouth (25 gm. infected placenta, finely chopped and suspended in salt solution) as well as through the conjunctiva (some drops of infectious suspension) and at different stages of pregnancy. All these heifers, excepting one, aborted after the infection.

TABLE II.

SURVEY OF INCUBATION PERIOD AND BLOOD REACTIONS IN PREGNANT HEIFERS INFECTED AT VARIOUS STAGES OF PREGNANCY.

| *Heifer No.* | *Interval between Copulation and Infection.* | *Incubation.* | *Interval between Infection and Presence of Positive Blood Reactions.* | *Agglutination Titre at Abortion.* |
|---|---|---|---|---|
| 200 | 10 days | 172 days | 16 days | 1–500 |
| 184 | 21 „ | 209 „ | 84 „ | 1–1,000 |
| 198 | 34 „ | 199 „ | 40 „ | 1–1,000 |
| 196 | 47 „ | Normal delivery | 40 „ | — |
| 197 | 62 „ | 121 days | 44 „ | 1–200 |
| 284 | 93 „ | 101 „ | 34 „ | 1–50 |
| 281 | 4 months | 112 „ | 24 „ | 1–500 |
| 287 | 5 „ | 80 „ | 34 „ | 1–500 |
| 185 | 6 „ | 67 „ | 54 „ | 1–1,000 |

After this experiment there is nothing surprising in the very long incubation period when the infectious material is transmitted by the bull through copulation. This experiment also illustrates the relativity of the incubation; besides, it helps to explain the most divergent statements in the literature concerning the length of the incubation period.

As will be noticed from Table II, the introduction of the infection in these pregnant heifers was followed fairly soon by positive reactions in the blood. On the whole, the reaction values were high, the highest being demonstrated at the time of the abortion or shortly after.

## CONCLUSIONS.

As *conclusions* that may be drawn from the experiments here reported, the following may be indicated:—

It is here established experimentally that the spread of abortus infection by the bull in the act of copulation is practicable in principle. The resistance of the cows against the infection at the time when pregnancy commences, however, is considerable, five of the infected heifers calving normally, in spite of having been exposed to such a massive infection. Hence, in practice, we have at present to reckon with the possibility that transmission of the infection by the bull may also take place under natural circumstances. Still, in view of the fact that the resistance of the cow is rather considerable at the time of copulation, and that the amount

of infectious material transmitted under natural conditions will always be far less than the amount employed in these experiments, it is only reasonable to assume that transmission of the infection by the bull is yet of minor importance in the spreading of infectious abortion—as has been claimed by several, especially American, investigators.

It is therefore still desirable to continue experiments on transmission of this infection through copulation with bulls that have been infected under "normal conditions," *i.e.*, bulls that have covered bacilli-excreting aborting cows and thus contracted a moderate degree of the infection.

## Summary.

Experiments were made in regard to the possibility of copulation infection of cattle with infectious abortion. These experiments were made with three bulls and 15 heifers, besides four control heifers. The bulls were infected strongly in the prepuce (suspension of infected placental material) immediately before they covered the heifers.

The result was that five heifers aborted, five calved normally, and five did not become pregnant. All four control heifers calved normally.

It has thus been demonstrated experimentally that copulation infection may actually take place, but it is emphasised that although the heifers were here exposed to massive infection abortion took place only in half of the pregnant heifers.

These findings presumably indicate a considerable resistance of cattle to this infection at the time when pregnancy commences.

The experiments may hardly allow of any decisive conclusions about the transmission of the infection by copulation under normal practical conditions, for it is only reasonable to assume that under normal conditions (leaving out specific orchitis and other genital lesions in the bull) the amount of infectious material that may be transmitted in the copulation will be far less than what was transmitted in these experiments.

Until additional experiments are carried out with more definite determination—titration—of the infecting capacity of the bull, we have to reckon with this possibility under normal conditions. It is therefore not practicable at present to relax the statutory regulations applying to cattle breeding in our country.

On transmission of the infection by copulation the incubation period will be very long—averaging 225 days in these experiments—and the reactions will as a rule appear very late.

In pregnant animals in which the infection takes place the incubation period will be inversely proportional to the development of the pregnancy.

TABLE I.

EXPERIMENTS ON THE SPREAD OF ABORTUS INFECTION BY MEANS OF INFECTED BULLS.

| *Heifer No.* | *Date of Copulation and Infection.* | *Serological Examination.* | *Date of Abortion.* | *Calving.* | *Remarks.* |
|---|---|---|---|---|---|
| 288 | 22/10, 1929 | 1/10, 23/10, 11/11, 3/12, 1929, 25/2, 28/3, 1930: Ag. 0, Cp. 0; 2/5 Ag. 0·05 p., Cp. 0; 30/5 Ag. 0, Cp. 0·02; 12/6 Ag. 0·005, Cp. 0·001 | 11/6, 1930 | — | 232 days of pregnancy. Abortus bacilli demonstrated in the afterbirth, where pathological changes were only slight. |
| 283 | 22/10, 1929 | Examined 10 times during pregnancy, each time with this result: Ag. 0, Cp. 0 | — | 27/7, 1930 | 279 days of pregnancy. Afterbirth normal. No abortus bacilli demonstrated. |
| 57 | 12/4, 1932 | 15/4, 27/4, 17/5, 11/6: Ag. 0, Cp. 0; 25/7: Ag. 0·05, Cp. 0; 27/8: Ag. 0·05, Cp. 0·1 p.; 1/10: Ag. 0·05, Cp. 0; 22/11 1932: Ag. 0·05, Cp. 0·1 | — | — | No pregnancy. |
| 159 | 19/4, 1932 | 27/4, 17/5, 11/6, 25/7, 27/8: Ag. 0, Cp. 0; 1/10: Ag. 0·05 p., Cp. 0; 22/11: Ag. 0·05, Cp. 0·05; 26/12, 1932: Ag. 0·002, Cp. | 26/12, 1932 | — | 251 days of pregnancy. Abortus bacilli demonstrated in the afterbirth, which showed pathological changes. |
| 58 | 25/4, 1932 | 26/4, 17/5, 11/6, 25/7, 27/8, 1/10: Ag. 0, Cp. 0; 22/11: Ag. 0, Cp. 0·02; 24/12, 1932: Ag. 0·01, Cp. | 20/12, 1932 | — | 239 days of pregnancy. Abortus bacilli demonstrated in the afterbirth, which showed pathological changes. |

Ag. — Agglutination. Cp. — Complement fixation. p. — partial.

TABLE I—(*Contd.*).

| *Heifer No.* | *Date of Copulation and Infection.* | *Serological Examination.* | *Date of Abortion.* | *Calving.* | *Remarks.* |
|---|---|---|---|---|---|
| 194 | 25/4, 1932 | Examined 8 times during pregnancy, each time with this result : Ag. 0, Cp. 0 | — | 31/1, 1933 | 281 days of pregnancy. Afterbirth normal. |
| 193 | 3/5 and 2/7, 1932 | 3/5, 17/5, 11/6, 2/7, 25/7, 27/8, 1/10 : Ag. 0, Cp. 0<br>22/11, 1932 : Ag. 0·02, Cp. 0·1<br>30/1, 1933 : Ag. 0·005, Cp. 0·02 | 29/1, 1933 | — | 211 days of pregnancy. Abortus bacilli demonstrated in the afterbirth, which showed pathological changes. |
| 157 | 7/5, 13/10, 1/11, 1932 | From 9/5 to 1/11, 1932, altogether 7 examinations, each showing : Ag. 0, Cp. 0 | — | — | No pregnancy. |
| 60 | 27/6, 1932 | From 27/6, 1932 to 1/4, 1933, altogether 6 examinations, each showing : Ag. 0, Cp. 0 | — | 1/4, 1933 | 278 days of pregnancy. Afterbirth normal. |
| 192 | 2/7, 2/8, 1932 | From 2/7 to 27/8, 1932, altogether 3 examinations, each showing : Ag. 0, Cp. 0 | — | — | No pregnancy. |
| 209 | 29/11, 20/12, 1932 | 19/11, 1932 : Ag. 0, Cp. 0 | — | — | No pregnancy. |

Ag. — Agglutination. Cp. — Complement fixation. p. — partial.

TABLE I—(*Contd.*).

| *Heifer No.* | *Date of Copulation and Infection.* | *Serological Examination.* | *Date of Abortion.* | *Calving.* | *Remarks.* |
|---|---|---|---|---|---|
| 33 | 22/9, 13/10, 1933 | 23/9, 13/10, 15/11 : Ag. 0, Cp. 0<br>16/12, 1933 : Ag. 0, Cp. 0·05<br>15/1, 1934 : Ag. 0·02, Cp. 0·02<br>14/2 : Ag. 0·02, Cp. 0·1 — 16/3 : Ag. 0·005, Cp. 0·05<br>17/4, 25/4 : Ag. 0·005, Cp. 0·005 | 24/4, 1934 | — | 193, days of pregnancy.<br>Abortus bacilli demonstrated in the afterbirth, which showed pathological changes. |
| 40 | 22/9, 1933 | Examined 10 times during pregnancy, each time with this result : Ag. 0, Cp. 0 | — | 9/7, 1934 | 290 days of pregnancy.<br>Afterbirth normal.<br>No abortus bacilli. |
| 36 | 11/10, 1933 | From 23/9, 1933 to 22/5, 1934, altogether 9 examinations, each showing : Ag. 0, Cp. 0<br>17/7 : Ag. 0·005, Cp. 0·01 | — | 16/7, 1934 | 278 days of pregnancy.<br>Calf normal. Afterbirth eaten by the heifer. |
| 191 | 23/11, 1933<br>3/1, 1934 | 15/11, 16/12, 1933 : Ag. 0, Cp. 0<br>15/1, 1934 : Ag. 0·02, Cp. 0<br>14/2 : Ag. 0·05, Cp. 0 — 16/3 : Ag. 0, Cp. 0 | — | — | No pregnancy. |

Ag. — Agglutination. Cp. — Complement fixation. p. — partial.

TABLE I—(*Contd.*).

CONTROL HEIFERS, PREGNANT WHEN PURCHASED.

| *Heifer No.* | *Date of Copulation.* | *Serological Examination.* | *Calving.* | *Remarks.* |
|---|---|---|---|---|
| 32 | 9/4, 1933 | Examined 5 times. No reactions throughout the experiment period | 14/1, 1934 | 280 days of pregnancy. Afterbirth normal. |
| 34 | 9/3 and 31/3, 1933 | Examined 5 times. No reactions throughout the experiment period | 18/12, 1933 | 262 or 284 days of pregnancy. Afterbirth normal. No abortus bacilli. |
| 35a | 28/5, 1933 | Examined 7 times. No reactions throughout the experiment period | 10/3, 1934 | 286 days of pregnancy. Afterbirth normal. No abortus bacilli. |
| 39 | 29/3, 1933 | Examined 5 times. No reactions throughout the experiment period | 2/1, 1934 | 279 days of pregnancy. Afterbirth normal. |

BULLS EMPLOYED IN THESE EXPERIMENTS.

| *Bull No.* | *Infected.* | *Serological Examinations.* |
|---|---|---|
| 1 | 22/10, 1929 | 23/10, 11/11, 3/12, 1929 and 2/1, 1930 : {Ag. 0 Cp. 0 |
| 2 | 12/4, 19/4, 25/4, 3/5, 7/5, 27/6, 2/7, 2/8, 13/10, 1/11, 29/11, 20/12, 1932 | 6/4, 14/4, 27/6, 2/8, 27/8, 1/10, 1932 : {Ag. 0 Cp. 0 |
| 3 | 22/9, 11/10, 13/10, 23/11, 1933, 3/1, 1934 | 23/9, 13/10, 28/11, 16/12, 1933, 15/1, 14/2, 16/3, 1934 : {Ag. 0 Cp. 0 |

# THE NATURE, EPIDEMIOLOGY AND CONTROL OF SHEEP MYIASIS IN BRITAIN.

## A Summary of Our Present Knowledge

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## I.—Introduction.

Although the maggot fly has been recognised for many centuries as a pest of sheep in Britain, it is only within the last few years that its activities have assumed such proportions as to constitute a serious menace to the sheep farming industry throughout the country. In Australia and South Africa, on the other hand, the problem of its control has been of foremost importance for many years. It is thus only natural that, until recently, practically all the important advances in our knowledge of the pest have come from these countries. Smit in South Africa, and in Australia a long succession of painstaking

investigators have accumulated a mass of information on the causes and methods of control of sheep strike.

In Britain, after the pioneer work of MacDougall some 30 years ago, the problem received little or no further attention at the hands of investigators until the beginning of the present decade, when the increasing ravages of the pest brought the question of its control into widespread prominence. It was soon realised, however, that much of the available information was not applicable to the problem as it existed in this country, and workers in many parts of Britain took up the study of different aspects of the investigation. The last few years have seen many additions to our knowledge of the nature and causes of the trouble, and of the habits of the flies concerned. These are scattered at present as more or less unrelated papers in several scientific publications, and it is the aim of the present paper to assemble the main features of this new evidence so as to present a more or less coherent account of the present position of the problem, more particularly with regard to its field aspects. An attempt will be made at a re-orientation of accepted ideas, where this appears to be indicated in the light of recent knowledge.

## II.—The Nature of Sheep Strike.

Strike, or cutaneous myiasis, is not a problem peculiar to sheep farming. " Maggots " in sheep is but one phase of a widespread form of insect activity—the common habit of many flies of living in their larval stages, on flesh. Different theories have been advanced to explain how the habit of striking living sheep arose. Froggatt[18] maintained that carrion-feeding flies, originally pure scavengers, had acquired a new habit of feeding in the course of time. Johnston[29], on the other hand, believed that there was no change of habit of the flies, and that the occurrence of strike depended on whether or not suitable attractive conditions were offered by the sheep. Wardle[51] suggested that the intense competition of other species in carrion, by driving the maggot flies to oviposit elsewhere, might be one of the factors causing the striking habit.

It seems to the writer unnecessary to look for an explanation of the strike habit elsewhere than in the normal carrion-infesting habit of the species concerned. To obtain a clear grasp of the situation, it is necessary to consider the question of cutaneous myiasis in animals generally. The problem of sheep strike—one aspect of this general question—will then be seen to fit logically into the scheme of things.

### *Myiasis in General.*

Reduced to simple, if somewhat teleological, terms, the occurrence of myiasis may be explained as follows.

The female fly instinctively knows that the eggs it carries must be laid on or near animal tissue if the carnivorous larvæ are to survive. It cannot recognise flesh by its appearance or size, but must rely

on certain odours which the experience of the race has taught are associated with the presence of suitable food for its young. As animal flesh decomposes the odours which emanate from it change in character ; different species of flies are attracted by different odours, and so there occurs in meat a definite succession of species until the food material has all been exhausted[25, 19]. It is obvious that the first species to colonise a carcase—those which arrive when the flesh is still fresh and even still warm—must do so in response to an odour or odours other than those of putrefaction. These odours will also be given off from the raw flesh and new-spilled blood of a wounded but living animal. It is through no fault of the fly, but because of the tenacity of life of the higher animals when maimed, that the tissue to which the gravid female is thus guided happens sometimes still to clothe a living man or beast. Whether the species learns by experience of the surprising success of such mistakes on the part of some of the more foolish or more daring of its members, is still a disputed point. It is an interesting fact, however, that in America a species of fly, *Cochliomyia hominivorax*, which normally colonises wounds, has occasionally been recorded as laying its eggs on the unbroken skin[30].

*Types of Myiasis.*

The infestation of fresh wounds as described above is known as wound-myiasis. There are two other kinds of myiasis which more nearly concern the sheep farmer. Some flies lay their eggs on either fresh or decomposing meat, *i.e.*, they are attracted by more than one combination of odours. The sheep maggot fly belongs to this group. It will strike a fresh wound, but it is also attracted by decomposition odours, and is thus led to oviposit on suppurating areas, such as foot-rot sores, and also on wool soiled with dung or urine and giving off ammoniacal or other decomposition odours. This fly will also oviposit on clean wool where there is no wound, and this has been held by some workers to prove their theory that the fly had changed its nature and acquired the habit of striking healthy sheep. Recent evidence, however, tends to show that this habit also is a response to decomposition odours. In Australia, it was found that body strike on unsoiled wool was always associated with decomposition processes, the most common expression of these being bacterial activity in areas of " water rot " of the wool. The increase of maggot fly in Australia need not, therefore, indicate a change of habit on the part of the fly, but a change of the type of host, by which stimuli for oviposition are more frequently presented to the fly than previously. In this country, it has recently been shown by Hobson[22] that any source of ammoniacal decomposition when placed on the wool of a living animal, will induce flies to lay eggs on that area.

Flies which initiate a strike on sheep, in response to attractive odours from a wound, sore, or decomposing matter, are called primary strike flies, to distinguish them from the secondary flies

which form a third group of myiasis-producing flies affecting the farmer. It has been pointed out above that as carrion decomposes its odours change. Now, the decomposition of carrion is largely the result of the activities of the larvæ in it. Some species of flies are attracted to meat which has been colonised and to some extent changed in nature by the larvæ of the primary species. It is probable that certain products of the activity of these larvæ contribute to the odour which attracts the later flies of the succession, and thus, when the host of the primary larvæ happens to be a living sheep instead of a dead carcase, it is not surprising that some of these later flies of a carrion succession, which would not be attracted to a sound sheep, are misled by the presence of odours they recognise into accepting what they think is their cue in the succession of flies in carrion. Such secondary flies cannot initiate strike, but can greatly increase the severity of an existing strike.

In severe or longstanding cases of strike yet other species of flies may be found. Such flies are known as tertiary flies ; though commonly occurring in carrion, they are rare in cases of strike in this country at least. In Australia, where struck sheep are necessarily under less close supervision than they are in the smaller flocks of this country, tertiary flies form an important group of the species involved in sheep strike. The attraction which operates in their case is unknown, but it is convenient to regard them here as belonging to the secondary group.

To summarise the above account—there are three types of flies which produce cutaneous myiasis in animals. Type A is attracted by the odour of fresh tissue and strikes wounds ; type B is attracted both by fresh and decomposing tissue, and strikes both wounds and unbroken skin ; type C is attracted by odours of decomposition only and can attack living animals only where there is already existing myiasis or diseased tissue. The three resulting kinds of strike-wound-myiasis, primary and secondary (including tertiary) myiasis, can be considered merely as variations of the carrion-infesting habit, and the high frequency of occurrence of these variations as the result of the abnormally high sheep population and of the artificial balance between living animals and dead carcasses maintained in the field under present conditions.

In Britain the first type of myiasis-producing fly is not represented, wound-myiasis, where it occurs being due to flies of the second group. Sheep strike is due to type B and possibly type C flies.

*A Survey of Myiasis in Different Parts of the World.*

A survey of myiasis shows that the three types are represented by different species in different countries. In America, two wound-infesting species, *Cochliomyia macellaria* and *C. hominivorax*, are present in the tropical and sub-tropical regions, and are responsible for practically all the cases of wound-myiasis of animals in the summer. One of these, *C. hominivorax*, seems to be a typical " type A " fly,

since in nature it appears to breed only in wounds of living animals. A very serious outbreak of myiasis by this fly occurred in 1933 and 1934, over one and a half million cases occurring in domestic animals in the South Eastern States of the U.S.A.[12].

Type B is represented in America by the common sheep maggot fly of this country, *L. sericata*, a closely related fly, *Lucilia cuprina*, and a black fly, *Phormia regina*. In the Southern and South Western States *Phormia* is, in the spring, the principal cause of myiasis, both on wounds and unbroken skin. In the summer and autumn, *L. sericata* and *L. cuprina* replace it as a sheep infester, while wound infestations of animals in general are now mainly due to *Cochliomyia* species[6]. The type C or secondary flies appear to be represented by species of *Sarcophaga*.

In Australia the problem presented by myiasis of sheep, although in existence only since the beginning of this century, has probably reached greater magnitude than anywhere else in the world. Among the undesirables sent out from this country to Australia in the early days of its colonisation, our maggot fly, *L. sericata*, was accidentally included, and at a later date *L. cuprina* was imported from the East. There were already four primary blowflies native to Australia, so that now the blowfly problem there is complicated by the fact that six primary species are concerned, in addition to four or more secondary species. An excellent account of the problem there was published in 1933 by the Council for Scientific and Industrial Research (pamphlet No. 37), and it is unnecessary to give further details here.

In South Africa, as in Australia, the blowfly problem is of recent origin, and is rapidly increasing in importance, but it has not yet reached such intensity as in the latter country. There are in South Africa two species of purely wound-infesting type A flies, *Chrysomyia bezziana*, which attacks cattle, and *Wohlfahrtia magnifica*, which has been recorded from man. The type B myiasis producers, at least in so far as sheep are concerned, are represented by the ubiquitous *L. sericata*, and an indigenous species, *Chrysomyia chloropyga*[16]. A third fly, *C. albiceps*, occurs in cases of strike to a less extent and mainly in the autumn. It is possible that the species may be a true secondary, *i.e.*, a type C fly. Type C is represented in the country by several species, but so far there are no records of their occurrence in cases of sheep strike.

On the Continent sheep myiasis apparently does not yet constitute a serious problem. *Lucilia sericata* occurs in France, and it has been reported as striking sheep in Holland. In Russia, although present, it has not been recorded as attacking sheep. Wound-myiasis in this and other animals is due to *Wohlfahrtia magnifica* and, in some areas, to *Lucilia cæsar*[37].

It follows from the argument advanced in earlier paragraphs that the myiasis-producing habit is a potential attribute of a group rather than a characteristic of a species. Therefore, the occasional appearance of any of the members of the types B and C groups in the

role of myiasis producers should not be a matter of surprise. Species other than those customary for a given country may be, and sometimes are, reported as infesting sheep, and, similarly, different species are sometimes reported as infesting unusual hosts. In China, *L. sericata* causes serious infestations of humans[36] and has been reported from cattle in different parts of the world[27, 46]; *L. cæsar* has been recorded from man[40], sheep[32, 37] and cattle[46]; the common blue-bottles, *Calliphora* sp. have been reported from sheep[10, 32, 39], man[8], and birds[44]; and a carrion-infesting species, *Phormia terræ-novæ*, has recently been recorded from cases of sheep strike in Scotland.

The myiasis habit is not, from the point of view of man, entirely reprehensible. In areas where foot-rot of sheep is common, the maggot fly is frequently found to strike the suppurating lesion, and many shepherds leave the larvæ in the wound, which they clean up more efficiently than would most antiseptic dressings. During the Great War, many of the wounded who were unable to receive immediate attention had their wounds struck by the maggot fly. It was observed that such wounds were less septic and healed more quickly than unblown wounds and from this observation there has been developed today an important form of wound treatment known as " surgical maggots." The method is peculiarly successful in the treatment of chronic bone infections, indolent wounds and ulcers. It has been mentioned previously that *Lucilia sericata* has the dual habit of blowing wounds and decomposing tissue. Brumpt[7] has suggested that there is a race of this fly which specialises in the first habit to the exclusion of the second. That is, according to the classification put forward above, the race belongs to the type A instead of the type B group of myiasis producers. The suggestion is made by Brumpt that this race could safely be used for surgical purposes, since its habit in nature precludes the likelihood of the larvæ carrying hereditary infection by septic organisms from carrion.

*The Species causing Sheep Myiasis in Britain.*

Stewart MacDougall, who studied the flies causing sheep strike in Scotland, found that *L. sericata* was the principal species, but that *Calliphora erythrocephala* occasionally occurred[32]. Later, Maldwyn Davies in Wales[10] found that, in 182 cases studied, all but two were due to *L. sericata* alone. In the two exceptions, *C. erythrocephala* occurred along with *L. sericata*. These two were fairly old cases and he suggested that *C. erythrocephala* acts in this country as a secondary fly. Ratcliffe[39], in Aberdeen, obtained this fly in only one case of strike, and does not exclude the possibility that it may have been an accidental contamination of his culture. He gives reasons for believing that *C. erythrocephala* can only act as a secondary striking species, and that it is unable to live on healthy or newly broken skin.

The writer has obtained five species of flies from cases of sheep strike in Scotland, *L. sericata*, *L. cæsar*, *C. erythrocephala*, *C. vomitoria* and *Phormia terræ-novæ*. The first three have also been obtained

from cases of strike in England. Further, strike has been produced experimentally on healthy sheep by *L. cæsar* and *P. terræ-novæ*, so that, whether they initiate strike in nature or occur only as secondary species, these perhaps should strictly be classed as primary flies, the definition of secondary flies including the essential inability of the species to originate strike. A full account of this work is being published elsewhere, and it need only be stated here that *P. terræ-novæ*, which so far has only been obtained from strikes in Blackface sheep in Argyll and Mull, appears to be an early summer fly ; the *Calliphora* species and *L. cæsar* are relatively uncommon and appear usually in the role of secondary flies, though *C. erythrocephala* has been obtained in pure culture and *L. cæsar* can occur alone in foot-rot sores ; *L. sericata*, which is by far the most important striking species of this country, occurs throughout the fly season, and is responsible for the great majority of body and breech strikes.*

These, however, are not the only flies concerned in sheep strike. The open sores left by the action of larvæ are frequently aggravated by the adults of other species of flies, principally *Fannia* species, which suck the blood and serous exudations, spread septic infection, and by the irritation of their presence and the consequent biting or rubbing on the part of the sheep rapidly extend the area of the wound. In lowland flocks especially these flies can be a serious source of trouble, for they cannot be controlled by the shepherd, and by keeping the wound open they leave the sheep continuously attractive to the maggot fly. The writer has seen cases of such sores, originally about an inch in extent, spread in a few days to an area of several square inches of raw skin, which in sunny warm weather were literally covered with a dense black layer of flies, their abdomens red with ingested blood. No dressing was found which would repel these flies and at the same time allow of the sore healing. They present a very definite problem in certain areas, and some lowland shepherds even go so far as to say that the real problem in mid-summer is not the activities of the maggot fly itself but the inevitable enlarging of strike lesions and constant harassing of the sheep from the irritation of the adult " black flies."

## III.—The History of Sheep Myiasis in Britain.

### *England and Wales.*

In England and the Borders of Scotland maggot fly attack seems to have been known for several centuries. The oldest reference to maggot fly appears to be that of Fitzherbert[15] in 1523, and frequent references to the trouble occur in the literature during the intervening centuries. Tusser, 1665[49], describes the symptoms and a means of control ; an anonymous writer[1] in 1683, and Ellis[13]

* Since this article was written an interesting paper by Haddow and Muirhead Thompson has appeared in *Parasitology*, 29, 96, recording the recovery of *L. cæsar*, *L. illustris*, *C. erythrocephala*, *C. vomitoria*, and *Phormia terræ-novæ* from sheep in South-Western and Western Scotland.

in 1749, refer to methods of control of maggots. Another anonymous writer, G.B.[3], writing in the Museum Rusticum in 1764, states, " The two greatest enemies the sheep . . . have, are the scab and fly." Youatt[52] was familiar with it in 1834, and Armatage[2] in 1895. It does not appear, however, to have assumed epizootic proportions at any time until the rapid increase of sheep farming and of intensive farming methods in the present century.

In Wales sheep rearing did not become an important part of the national economy until much later than in England and the Borders, and consequently the problem of maggot fly attack did not occur to any extent. It is not mentioned in the Ancient Laws and Institutes of Wales, 1841, which refers to different diseases of animals, but there is a reference in 1870-71 to its prevalence on low-lying farms in the North of Wales[47].

*Sheep Myiasis in Scotland prior to 1930.*

In Scotland sheep farming on an extensive scale was introduced into the Highlands from the Border country in the second half of the eighteenth century. It is probable that the maggot fly problem was unknown before the Cheviot and Blackface sheep supplanted the sparse flocks of the old native breeds. Thus, no mention of maggot fly appears in the voluminous writings of Robert Wight, who studied the farming conditions in the Highlands in 1772-1778, or in any of the agricultural writings of that period dealing with Highland conditions, to which the writer has had access.

From the beginning of the nineteenth century, however, sheep myiasis has been a serious problem in Scotland. It appears to have increased by a succession of epizootics, each of these leaving the problem more acute than it had been before. The first of these outbreaks was about 1826-27. In the records of the Plinian Society of the University of Edinburgh for 1827 there occurs a minute of a paper on the blowflies of sheep in the Scottish Highlands. It is recorded that, on five farms near Callander, of a total stock of 4,090 sheep, 550 were attacked and 195 died in the summer of 1827, and the curiously familiar statement is made that " the disease is annually spreading over the country and becoming more alarming." Confirmation of this outbreak is afforded by a shepherd of that time, one, William Hogg, of Peeblesshire, who wrote an essay in which he refers to the bad fly seasons of 1826 and 1827[24].

Subsequent to this outbreak, the trouble, though more widespread than before, apparently became relatively unimportant again. Occasional references to it appear throughout the rest of the century[34, 45, 48] but no further important outbreak seems to have occurred until about 1897-1900. This epizootic was common to the whole country ; MacDougall[31] quotes statements from farmers in practically every area of Scotland to the effect that the incidence of strike had increased to serious proportions only in the last few years of the century. In quite a number of these statements it was also

claimed that the fly had only recently been known to strike sheep in the areas in question.

This apparent expansion of the area of distribution simultaneously with each outbreak is an interesting feature of the epidemiology of sheep myiasis. Writing of the 1826 outbreak, the Peebles shepherd, Hogg[24], mentions that the evil was new to the shepherds, who were thus caught unprepared, and similarly a Dumfriesshire writer in 1839 states that maggots were worse in that area since 1826[45]. The 1897-1900 outbreak resulted in a similar extension of the affected area. Thus, in Ardnamurchan, dressing of struck sheep was a daily task in summer at least 80 years ago, while in Morvern, a comparatively short distance away, the maggot fly was unknown until about 1900, when a very severe season was experienced. Subsequent to this epizootic, cases remained comparatively rare in this area until recent years.

In Mull cases of strike were known only as isolated instances until 1930, and in Shetland the pest was almost unknown until quite recently. Ratcliffe[38] states that the pest has been advancing in recent years in Aberdeenshire.

*The 1931-34 Outbreak.*

During the years 1931-34, and particularly 1933-34, there was a very marked increase in the severity of strike and in the extent of the areas affected. On one farm in Mull the number of cases in 1932 was about double that of 1931, and the resulting mortality was about 5 per cent. of the annual death rate. In the following year the death rate rose to 8 per cent. and in 1934 to 35 per cent. of the total death rate, which, from louping ill, braxy and other causes, may be as much as 25 per cent. of the stock. Cases in this year occurred earlier than usual in most districts, and were found on the highest hill tops, which had previously been free. Another farm in Perthshire, of which the writer had personal experience, with a stock of about 3,000, was 500 head short of the May count at the September sales, the loss being attributed entirely to death from maggots.

Ratcliffe gives a number of estimates of the loss due to maggot fly, obtained in 1934 from farmers throughout Scotland. These vary within very wide limits, but if the average of all the given figures is taken, it is seen that the loss over the whole country comes to over a shilling per head of adult stock.

In comparison with the 1934 outbreak, the incidence in 1935 and 1936 appears to have been comparatively low in many places, but some districts report very serious losses.

A common feature of both the 1900 and 1934 outbreaks was the occurrence of strike on high-lying ground previously free from this trouble. This tendency for the fly to invade upland grazing during epizootic seasons is clearly brought out in the replies to questionnaires quoted by MacDougall in 1904 and Ratcliffe in 1934.

The causes of these epidemics and of the more gradual spread

during the intervening years are still obscure. No helpful indication can be obtained from a study of the events in other countries. Thus, in Australia, the spread of the trouble would appear to be due to (*a*) the introduction of new species of flies ; (*b*) the change of sheep breed, resulting in a more susceptible type ; (*c*) the change of environment of the fly, through the progressive destruction of bush and conversion of wasteland, and (*d*) possible changes in the sheep themselves through depletion of mineral reserves of the land and other food changes. In South Africa, the problem seems clearly to be a result of the introduction of Merino sheep. In America sheep myiasis is not generally a serious problem, but has recently become important in some States. The States affected are those which have introduced the highly susceptible Merino type of sheep. So far as Britain is concerned, there has been no introduction of a new species of fly, and in the areas where the pest has been definitely spreading during the last 80 years no change of sheep breed or outstanding improvement of an existing breed. The extent to which climate affects fly numbers is not yet known, and no theory which explains the change in terms of climatic causes can at present be supported.

## IV.—The Bionomics of *Lucilia sericata*.

### *Life History.*

The life history of the maggot fly is, in its essentials, familiar to every biologist and may be briefly dismissed. A summary of the physiology of this species has recently been published by Evans[14]. The time taken for development of the different stages varies considerably according to whether they are developing on a living sheep or on carrion ; the following summary is concerned only with the life history of individuals living as larvæ on sheep.

The eggs are laid in the wool in batches of 50 to over 100, the process of oviposition taking up to half an hour. They hatch in under 12 hours, the time varying by a few hours according to the proximity of the eggs to the skin, the depth and type of the fleece and the weather conditions. The newly-hatched larvæ move down to the skin surface, and, by the irritation of their presence and feeding, produce an inflammatory reaction which gradually develops to a raw sore, on the exudations of which the larvæ feed. Two moults occur, the first in about 12 hours and the second in about 24 hours[10]. In summer the third instar has completed its feeding by the end of the second day and crawls out of the fleece to the ground. There it enters the soil, and after a wandering period, the prepupal period, of usually three or more days, forms a puparium within which it pupates. The wandering phase is a remarkable feature of the life-history and may last for months according to the soil conditions in which the prepupa finds itself. The prepupa may travel for several feet through the surface layers of soil before finally pupating. In this country hibernation occurs in the prepupal stage[10]. Under ordinary summer conditions the adult emerges from the puparium after a fortnight

or so, and seldom in under eight days, although in exceptional cases the period may be as short as three days.

The female must obtain a meal of protein before its ovaries can develop, and if meat be withheld for more than ten days they will not develop subsequently[9]. Fertilisation occurs and the female commences, some five or more days after emergence, to lay eggs. Oviposition is repeated at intervals of 2 to 3 days, and a female may lay over 2,000 eggs in its lifetime. It is probable that many of the later eggs of a long-lived female are unfertile, but Salt[41] records fertile eggs from a 66-day old female. Under laboratory conditions the female may live for three months ; Salt records a maximum longevity of 104 days. In Britain four successive generations of flies may occur in a season[10, 50].

*Habits.*

*Lucilia sericata* can travel long distances, moving mainly downwind. It has been shown in Australia to be capable of travelling at least four miles in 16 days[20]. It likes sunshine but dislikes wind ; thus the open areas of grazings near the shelter of woods are favourite haunts. The rank vegetation of hedge bottoms, and, in hill country, bracken and heather, provide ideal shelter. The fly is most active in the forenoon and early afternoon, when the sunlight is at its maximum intensity. It is usually much less active on dull, cloudy days, even though these be warm and windless.

*Controlling Factors.*

All present day theories on the factors limiting the maggot fly population are based on the assumption that the main part of the population is reared on carrion, the proportion feeding on living sheep having a negligible effect on the total. How far this assumption is justified is a question on which there is little or no evidence. It has been shown by Holdaway[26] that parasites and predators do not affect the ultimate numbers of a population of blowfly larvæ until the main bulk of that population has been destroyed by the competition of other species. In so far as carrion is concerned, he has been able to show that the effect of competition is of extreme importance. Under summer conditions, of an initial population of 50,000 *L. sericata* in an exposed bait the final emergence was 231 adults. In another experiment an initial population of 60,350 was so ravaged by competing species that only 30 survived.

Salt[41] has suggested that the competition between individuals of the same species is as important a controlling factor as interspecific competition, *L. sericata* so heavily overblowing the available meat that only a few of the resulting larvæ are able to obtain sufficient nourishment to complete their development. It is obvious that neither of these factors operates with comparable intensity in the case of a larval population of a strike lesion.

James[28] maintains that parasites are responsible for a reduction of 30 per cent. in the blowfly population in England. The suggestion

that parasites and predators may materially affect a blowfly population is also contained in the observations of Baudet and Nieschulz[4], who state that extensive striking occurred in 1932 on the newly-reclaimed areas of the Zuider Zee in Holland, while the neighbouring old polders were relatively immune from strike. It is supposed that the phenomenon was connected with the absence of the natural enemies of the maggot fly from the newly-reclaimed land.

The effect of change of edaphic factors, through improvement of land or reversion of cleared land to waste, has not been properly investigated. In the Scottish Highlands it is generally maintained that the spread of bracken is one of the chief causes of the increase of maggot fly. Other possibilities which deserve consideration and on which there is very little evidence, are the effect of climate, *e.g.*, abnormal winters and summers, the effect of changes in the density of population of deer, rabbits and such, and the possibility of a fundamental relation between population level over a period of years and the general post-war trend of agriculture in the direction of higher densities and wider distribution of sheep flocks throughout the country.

## V.—The Factors Affecting the Occurrence of Strike.

Three main conditions are necessary for the development of strike on sheep: (*a*) suitable weather for fly activity, (*b*) a source of attraction to the adult fly on the body of the sheep, this attraction including the necessary stimuli to oviposition, and (*c*) suitable microclimatic conditions in the fleece to allow of development and hatching of the eggs and survival of the larvæ. These three involve the operation of factors which may be divided into two groups, host factors and climatic factors. The host factors include age, breed, individual susceptibility and microclimatic conditions in the fleece, the last being also affected by climatic factors.

### *Host Factors.*

It is well known that the different breeds vary in their susceptibility to strike. The Merino sheep appears to be exceptionally susceptible, and it would appear that the nearest approach to it in this country, the Corriedale, is similar in this respect. An opportunity of observing the relative susceptibility of different breeds was secured during the course of some experiments in 1935. The strikes in a mixed flock running together on a field near Stirling were noted during the month of June. At shearing time, four weeks after the commencement of the experiment, the results were as follows :—

| *Breed.* | *No. of Sheep.* | *No. of Strikes.* | *Percentage* |
|---|---|---|---|
| Corriedale ... ... ... ... | 16 | 8 | 50 |
| Lincoln... ... ... ... ... | 15 | 4 | 28 |
| Romney Marsh ... ... ... | 30 | 5 | 17 |
| Shropshire ... ... ... ... | 14 | 2 | 14 |
| Halfbred and Greyface ... ... | 29 | 2 | 7 |
| Shetland ... ... ... ... | 20 | 0 | 0 |

Miller[35] mentions the apparent immunity of the Shetland breed, and found that brown-woolled sheep in general were relatively immune. It does not appear, however, that this immunity is due simply to the dark colour, for the writer has repeatedly produced strike experimentally on a black-woolled Hampshire and has obtained natural blow on the same animal.

Maldwyn Davies[10] found that the Wiltshire breed was markedly insusceptible, and the Border Leicester comparatively so, whilst the Southdown was highly susceptible. The Welsh breed appeared to occupy an intermediate position.

In an experimental flock under the writer's control, consisting of Hampshires and crosses between half-bred ewes and Hampshire and Suffolk rams, the number of strikes in proportion to the number of " sheep-days " for each breed, *i.e.*, to the product of the number of sheep exposed and the number of days of their exposure, was as follows : Hampshires, 1 : 50 ; Hampshire crosses, 1 : 130 ; Suffolk crosses, 1 : 103. Thus it would appear that the introduction of Cheviot or Border Leicester blood lessens the susceptibility of Down breeds to strike.

So far as the two principal hill breeds in Scotland are concerned, there is said to be a greater susceptibility in the Cheviot than the Blackface[31]. It is difficult ordinarily to get comparative data on this question, because of the fact that the compared flocks are usually on different farms or pastures. An opportunity was obtained, however, to get a true comparison on a farm in the Pentland Hills, where the Cheviot stock was gradually being replaced by Blackfaces. At the time of inspection (August, 1934), the breeds were roughly equal in numbers and running together. An inspection was made of approximately 100 of each breed, and the number showing evidence of recent strikes noted. Out of 85 Blackfaces 7 showed marks,—over 8 per cent., whereas in 112 Cheviots 3 showed marks—under 3 per cent.

As regards the age factor, it is difficult to assess the relative susceptibility to strike of lambs and adults, because of the complicating fact that seasonal variations in their susceptibility do not occur simultaneously in both age groups. Thus, the adults are susceptible before shearing when the lambs do not generally carry enough wool to allow of strike developing, whilst the period immediately after shearing is, for the adult sheep, relatively free from strike. Maldwyn Davies[10] found a greater incidence of attack in Wales among lambs than in older stock. MacDougall[31] states that in Scotland lambs were more frequently struck than adults. It is doubtful, however, if this is always the case among hill flocks. In Argyll, some shepherds state that, until recently, strike among lambs was practically unknown, the trouble being confined almost entirely to adult sheep. The writer's own experience has been that strikes, over the whole season, tend to be more numerous in the adult than in the lamb stock in so far as hill farms are concerned.

It is generally recognised that differences exist in the susceptibility of individual sheep of the same breed. Hobson[21] has shown experimentally that the attraction of sheep for blowflies varies considerably between one animal and another, and it is a common observation in the field that certain animals are repeatedly struck. Such restrikes, however, are not always so common as to affect markedly the total incidence of strike in particular groups. Ratcliffe[39] from a study of the Rowett Research Institute flocks, found that restrikes formed a low proportion of the total number of cases occurring. This does not apply, of course, to strike on the soiled rump or breech of sheep. Repeated strikes of this type may occur on the same sheep, not necessarily because it is inherently susceptible but because of the persistence of scouring or other cause of the soiling of its hind quarters. In so far as the question of inherent susceptibility is concerned, only body strikes should be taken into consideration, and there are no precise data of this type yet available from the work of other investigators. An indication that the effect of individual inherent susceptibility on the general incidence of strike is low may perhaps be drawn from the following limited data. Fourteen natural cases of body strike, in an experimental flock of 60 lambs, were obtained in 1936 ; of these only three were repeat strikes, on new areas of the same animal, *i.e.*, the 14 cases were distributed over 11 sheep.

Apart from the question of an inherent susceptibility of certain individuals, it is now known that strike is not a matter of chance, but is due to the development of an area of susceptibility. This area may become susceptible through soiling with moist fæces and urine. Another case of the development of susceptibility is the occurrence of " scalding," or pruritis of the unprotected skin of the perineum. This condition is commoner in lowland sheep, and especially in fat breeds, than in hill flocks. It appears to occur most commonly in hot weather before the sheep are relieved of their fleeces. These two causes, soiling and scalding, are probably responsible for most of the breech or rump strikes which occur.

In Britain, however, cases of strike on clean wool—body strikes—form a fairly large proportion of the total number occurring. The host factor in these cases is still unknown. In Australia, it has recently been suggested that the suint content in the fleece is correlated with susceptibility[26]. This theory is not, however, supported by the researches of Hobson[23], who was unable to find any such correlation in so far as Welsh sheep were concerned. Water rot, the result of bacterial activity in the wool, has been shown invariably to precede body strike in Australia[43]. In this connection, Beveridge[5] has made the curious observation that water rot is more frequent in shorn lambs than in lambs of the same breed whose fleeces have been left long.

It is of interest that, so long ago as 1835, practical sheepmen recognised the fact that susceptibility to strike was an abnormal condition, and held the belief that yolk content and yolk coloration

were related to susceptibility. George Mather, a Perthshire shepherd, in an excellent contribution to the Prize Essays of the Highland Society[34] made the following observations, which might well have been written at the present day : " Maggots cannot breed on sheep unless there be an accumulation of perspired fluid. . . . The perspiration is found lodged at the root of the fleece, and on the verge of this damp or sweat the fly deposits its eggs. Some individuals have this perspiration thicker and of a more yellow colour than others, and are on this account more subject to maggots."

In this country a common cause of body strike is the presence of extraneous organic matter in the fleece. The origins of these areas of soiling may be varied ; one of the commonest is the presence of putrefying larvæ in the fleece, as a result of the dressing of a previous strike with a rapidly toxic larvicide. Blood staining from abrasions, cuts, or from the bursting of engorged ticks, have also been reported as causing strike.

There is another possible cause of strike, in the Highlands at least. Strike in the neck, although generally a very rare occurrence, is relatively common in Western Scotland. One farmer states that attack about the neck is a common feature of strike in Mull. In Western Scotland in 1936, out of eight observed body strikes occurring between July 1st and July 8th, five were on the neck. The average hill grazing in the west is overrun with bracken, and in forcing their way through patches of this heavy growth sheep frequently get bracken fronds entangled in the wool of the neck and shoulders. It is possible that the decaying bracken may act hygroscopically in wet weather and form a more or less deliquescent slime, as it sometimes does when cut before it has matured. Such a source of moisture and organic decay might well be expected to be highly attractive to flies.

*Climatic Factors.*

Climatic factors assert their influence on strike by affecting the activity of the adult fly, the susceptibility of sheep to blow, and the suitability of the fleece for establishment of strike. Little exact information on the relations between climate and fly activity is so far available. Sunshine, warmth, and the absence of wind seem to be necessary conditions for activity. A possible role of sunshine is that of raising the temperature of the insect by insolation, when the air temperature is below that favourable for activity. This may be the explanation of the fact that oviposition was obtained on sheep in the autumn of 1936 on days when the maximum reading in the screen was 11°, 12° and 13° C., a fly being actually observed in one instance ovipositing when the air temperature was 13° C. Normally, oviposition occurred only on days when the maximum temperature exceeded 16° C.

It is usually stated that high humidity is also necessary for activity, but this impression is based on a confusion of strike with blow ; strikes occur more frequently after damp weather than during

dry spells, *ergo*, the fly strikes more frequently in damp weather. It must be borne in mind, however, that fly activity results in the blowing of sheep, and it by no means follows that such sheep will develop strike. This will depend on whether or not conditions in the fleece are suitable for survival of eggs and larvæ. These conditions are usually more suitable during, and immediately after, wet weather than they are in dry weather. Therefore, although strike after wet weather indicates recent fly activity, the absence of active strike in dry weather does not necessarily mean absence of fly activity. Failure to appreciate this distinction has resulted in the frequent statement that high atmospheric humidity in addition to warmth favours activity of the maggot fly. Actually, there is no direct evidence on this point.

Climatic factors will affect the development of strike from blow by modifying the general microclimatic conditions in the fleece. Maldwyn Davies and Hobson[11] have produced evidence to suggest that the moisture conditions in the fleece are normally unsuited to survival and development of larvæ, and this suggestion is supported by the writer's observations during the last season. Thus, out of 64 instances of blow kept under observation between June 20th and August 29th, 1936, only 29 developed to active strike, despite the fact that the period was abnormally wet, only 19 days out of the 70 being without appreciable precipitation. The rate of drying of rain-sodden fleeces will depend largely on such climatic conditions as wind, sunshine and humidity. On pastures with a rank growth of vegetation, as for example bracken, long heather, rushes or bent, heavy dew or mist will result in the fleeces becoming saturated through brushing against the moisture-laden vegetation.

The individual susceptibility of sheep is also affected by climatic conditions. Thus, rainscald will develop in some sheep in wet, warm weather and render them attractive to the fly ; hygroscopic organic matter in the fleece, such as dead larvæ, and possibly decaying bracken fronds, will absorb moisture in wet weather and form sites of attack. Climatic conditions generally, by affecting the succulence of the herbage, probably determine to some extent the expression of such tendencies as scouring, excessive skin secretion, and other reactions on the part of abnormal sheep.

## VI.—The Types and Development of Strike.

### *Body Strikes.*

As has been mentioned previously, body strikes form a large proportion of the total strikes occurring in this country. During the years 1934-36, the total number of strikes under the writer's personal observation amount to 120. To these may be added 40 cases of which particulars were obtained from Western Scotland in 1936. Of the 160, 100 were breech and rump strikes, and 60 occurred on various parts of the body. The distribution of these two major categories shows some interesting variations. Thus,

of 83 cases in Blackface and Cheviot sheep 40 were breech strikes and 43 body strikes, whilst of 77 cases in lowland flocks 60 were breech strikes and only 17 were body strikes. Of the 40 reported cases in Blackfaces 16 occurred in long-woolled sheep, 14 in clipped sheep and 10 in lambs ; of the 16 long-woolled cases 8 were breech and 8 body strikes, whilst 11 of the 14 cases in shorn sheep and 7 of the 10 in lambs were body strikes. Ratcliffe[39] makes a similar observation ; he found that, whereas the early season strikes on long-woolled sheep were mainly in the breech, later strikes, mainly on lambs, occurred to a great extent on the body. This is a surprising result, for one would have expected that sheep carrying a heavy fleece would be more susceptible to blow on the body, and would provide more favourable conditions for development of strike than would short-woolled sheep.

Body strikes have been found to occur mainly on the shoulders, including the withers. A lesser proportion occurs on the loins, and the remainder includes strikes on such areas as the neck, throat, chest, abdomen, scrotum and ribs.

*The Development of Strike.*

Under hot summer conditions, and provided the fleece conditions are suitable, development of strike can be almost unbelievably rapid. The larvæ may attain to full growth and drop from the sheep within two days of hatching. In the course of their feeding these will have produced typical strike lesions and staining, and these areas will have been heavily blown. Thus, clean unstruck sheep may in a matter of four or five days be covered over practically the entire surface of the body with larvæ, every nest formed becoming the focus for further strike. In hot weather, where the fly is numerous, healthy sheep may be killed within a week as a result of the rapid extension of strike.

The type of strike which develops depends on the fleece conditions. If the source of attraction is a moisture-holding material, such as a tag of soiled wool, the larvæ will tend to remain more or less together, and the skin may be broken at an early stage. If the strike is on clean wool, there is a greater tendency for a scattered type of strike to develop, with numerous small pockets of larvæ, and sometimes no serious skin damage. In short-woolled animals, such as lambs and recently-clipped sheep, the skin is usually broken fairly early, the moisture conditions in the fleece being unfavourable for surface migration. With the same species of fly, the type of strike varies widely according to the density and depth of wool, the origin of the strike and the microclimatic condition in the fleece. This is the chief objection to the argument commonly advanced by shepherds that there must be more than one species of fly concerned since different kinds of strike occur.

## VII.—Seasonal Changes in Strike Incidence.

Strikes have been obtained as early as April and as late as November, but these are exceptional instances, and the maggot fly problem is usually acute only from the beginning of June to the end of September. During this period there occur four phases of strike incidence, the limits of which depend on the type of sheep farming practised. In the Highlands there occurs a first phase during the month of June. At this time the lambs are not yet susceptible, and practically all the strikes occur in the unclipped adult stock. This phase is terminated by the clipping, which takes place usually at the end of June or early in July. There follows a phase of relative freedom from strike, since neither the adults nor the lambs carry a sufficient coat to allow of body strike being prevalent. Towards the end of July the occasional cases occurring in the lamb flocks begin gradually to increase in number as the lambs become more susceptible. This phase also shows, latterly, the growth of the late-season strike-incidence in adults. The fourth and last phase is represented by the September strikes. These occur in both adults and lambs, since both are now susceptible. This phase lasts until the onset of cold weather ends the fly season.

The incidence of strike over the whole flock will thus be usually in the form of a bimodal curve, the abruptly truncated first part of which represents strike in long-wool adults, and consists largely of breech strikes. The trough is the period of comparative freedom in July and the second elevation is due mainly at first to lamb strikes, and latterly to both lamb and adult strikes. In comparison with the first wave of activity, the strikes in the second period consist in a large proportion of body strikes.

This sequence is illustrated in the distribution of the Argyll cases previously referred to, except that the fourth phase of activity is poorly represented, the weather having been exceptionally unfavourable. Excluding tup strikes, there were 38 cases ; of these, up to shearing time in early July, 16 occurred in ewes and two in lambs (phase 1) ; from shearing to August 16th (phases 2 and 3), two cases occurred in ewes and six in lambs, whilst between August 16th and September 9th, nine of the cases occurred in ewes and three in lambs.

## VIII.—Control Measures.

### *The Possibilities of Control.*

Before discussing recent advances in methods, it is appropriate here to consider the general question of the possibilities of control. If the theory advanced in the early part of this paper is sound, the species *L. sericata* is not peculiarly the enemy of the sheep, but merely happens to be that carrion-infesting species whose particular range of attracting odours is most frequently encroached upon by modern sheep breeds under present day conditions. Other days, and new developments in sheep rearing, might possibly result in

another species becoming the paramount striking fly. Logically, it should be wise to concentrate, not so much on attacking the fly, which is but one of a host of potential enemies, as on protection of the sheep from attack. The argument is sometimes advanced that if all sheep are rendered unattractive, the flies, not finding ideal conditions for oviposition, will become less fastidious in their choice and will attack relatively unattractive sheep. This argument overlooks the fact that living animals are not the sole, and probably not the principal, source of food of the larval stages of the fly. The maggot fly population is so vast that it is doubtful if the number of larvæ which succeed in feeding on living sheep will very markedly affect the population level. This further suggests the advisability of concentrating control efforts on protection of the sheep.

Control measures in general may be divided into three groups—biological, mechanical and chemical. The greatest advances have been made in the first, and to a less extent in the third of these categories but, unfortunately, they do not apply to the sheep breeds and conditions in this country so much as to Australian conditions. The recent discovery of methods of ensuring blow[22] and the elaboration of a method of producing strike experimentally[33] give hope of early advances in the improvement of chemical means of control.

*Biological Methods.*

Efforts at control by interference with the balance of numbers between maggot flies and their parasites have not been attended with much success. The fact that maggots feeding on living animals are seldom, if ever, parasitised, militates against the chances of complete success of this method, and a further serious drawback lies in the large number of closely related carrion-infesting species, so that the weight of attack by the parasite tends to be dissipated over larval populations largely composed of relatively harmless species. Much more encouraging results have accrued from work on modification of the host. Seddon and his co-workers in Australia[43] have shown that the tendency to susceptibility is to some extent an hereditary character and that by selective breeding a sheep stock can be obtained which has a conformation less adapted to the retention of moisture in skin folds and consequently less liable to develop areas of susceptibility.

Another method of modification of the host successfully used in Australia is by surgical operation. Thus, Mules' operation corrects by surgical methods an excessive tendency to urine staining. Malformation of the tip of the vulva as a result of a shearing wound is another cause of urine staining and can be corrected surgically.

*Mechanical Methods.*

Mechanical methods include trapping of the flies, burial of carrion, and also the physical modification of the microclimatic conditions in the fleece. The value of trapping is rather a controversial point, many practical men holding that where properly

practised, it results in a marked reduction in the incidence of strike, while other workers hold that it is of little value and may even be detrimental, because of the large numbers of competing flies which are killed by the traps. It should be stated, however, that there is no experimental evidence in support of the contention that trapping is inadvisable. Traps capture large numbers of *Calliphora* species, and these are the principal alternative species of larvæ in carrion in this country. These larvæ do not actively interfere with *L. sericata* maggots, apart from the " overstocking " effect which operates impartially on both species. In any case, this species itself, as has been mentioned above, occasionally strikes sheep. Thus the destruction of large numbers of *Calliphora* flies is not necessarily a disadvantage. Ratcliffe[38] has shown that only about 5 per cent. of the flies caught in traps are *L. sericata*. However, since very many thousands of flies can be caught by systematic trapping, this percentage should represent a very considerable number of maggot flies in the course of a season.

A method of trapping which might with advantage be employed more extensively than at present is that of hanging rabbit or other carcases over running water. Alternatively the carcass may be suspended over a container kept full of toxic solution. Clearly, however, before trapping can make any great headway as a practicable control measure, a chemical bait is required which has a specific attraction for the maggot fly. Hobson[21, 22] has shown that ammonium carbonate, indole or the excreta of the larvæ themselves are all highly attractive to *L. sericata*, but this attraction is only effective in the presence of live sheep.

The fact that if the fleece be kept dry the tendency for strike to occur is reduced has long been recognised, and is made use of in the custom in certain areas of giving the sheep " rubbing scaurs," or sandy banks on the hillside. The sheep rub themselves against these banks, and the sand thus introduced to the fleece tends to make the fleece conditions unfavourable for survival of larvæ. Maldwyn Davies[10] cites a very interesting record of the empirical application of this result in the writings of Thomas Tusser in the sixteenth century, in which the application of dust to the affected part is advised.

In so far as the disposal of carrion is concerned, it is safer to bury all dead animals or offal at the earliest possible opportunity, rather than to wait for them to become blown. It is seldom that a carcase is buried sufficiently deeply to prevent any larvæ in it from returning to the surface when fully fed. Larvæ have been known to pupate 6 feet below the surface, and adult flies, newly emerged from puparia, have reached the surface from depths of over 4 feet[46]. Another reason why carcases should immediately be buried is that a protein meal is necessary for maturation of the ovaries of the newly emerged female, and if the meal is not obtained in the course of the first ten days of adult life the female remains sterile. A reduction in available carrion

should, therefore, affect the fly population both directly through the larvæ and indirectly through the females.

A method of mechanical control, which may have limited possibilities under certain conditions, has been suggested by Maldwyn Davies[10]. The hibernating prepupæ move upwards in the soil to the top layers in March and April in order to pupate. Where sheep have been closely folded during the previous strike season, as for example on green crops, and where consequently there should be a high larval population in the soil, cultural methods at this season might be adopted to kill the pupæ before the adults emerge.

*Chemical Control.*

Much work has been done abroad on the search for the most suitable type of dressing for struck sheep. The recent work of Freney and his collaborators in Australia[16, 19] on the use of glycero-boric preparations represents a very distinct advance. In these dressings a larvicidal substance, boric acid, is liberated from the dressing by the exudation of the lesion produced by the larvæ. The dressings have excellent healing properties, and a marked, though not absolute, protective value against restrike. Unfortunately, however, they possess, under the climatic conditions of this country, a distinctly disadvantageous feature. Larvæ killed by the dressing are prevented from desiccating, probably as a result of the glycerine present, and putrefy in the fleece. The presence of hygroscopic and sometimes semi-liquid putrefying larvæ results in repeated blow on the area in question and ultimately, as the toxic principle becomes exhausted, restrike succeeds in becoming established.

In connection with the treatment of struck sheep, the point has been stressed by both Maldwyn Davies and Ratcliffe that the great majority of the larvæ rubbed out of the fleece are capable of developing into adults, and it is pointed out that strikes should, therefore, be dressed with a larvicide before the maggots are removed.

The outstanding measure of control in this country still remains that of dipping. Jetting has not been practised to any extent and spraying as a method of control has only recently received much attention.

It is well to understand that the success of a dressing does not depend entirely on the manufacturer, but on the proper collaboration of the manufacturer and user. There is in practice an inevitable limit to the duration of the protection conferred by any chemical dressing, be it applied as a dip, spray or hand dressing. The growth of the fleece, gradually interposing a layer of new wool between the skin and the impregnated part of the fleece, the exhaustion of volatile repellent substances by virtue of their very efficacy, and the leaching effect of weather, all contribute towards the unavoidable termination of the period of protection conferred by the dressing used.

As indicated previously, the normal course of events, in hill flocks at least, is as follows: a first phase of maggot fly activity,

ending at shearing time, is followed by a more or less complete hiatus for a few weeks and subsequently by nearly two months of renewed activity. If, therefore, a system of three dippings each season were practised in the case of hill flocks, the first being carried out a month before shearing, say in early June, and confined to the ewes, the second at the end of July and including the lambs, and the third at the end of August, a high degree of protection over the season should be obtained. In lowland flocks, where shearing is usually over early in June, the dippings might be carried out at about the beginning of July, August and September.

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## References.

[1]Anonymous. (1683). A Treatise of Oxen, Sheep, Hogs and Dogs, etc. Obadiah Blargrave, London.
[2]Armatage, G. (1895). The Sheep Doctor. Frederick Warne & Co., London.
[3]B—, G—. (1764). *Museum Rusticum et Commerciale*, **3**, No. 16, 369.
[4]Baudet, E. A. R. F., & Nieschulz, O. (1933). *Abstr. Vet. Bull.*, **4**, 471.
[5]Beveridge, W. I. B. (1934). *Aust. Vet. Jr.*, April, 1934, p. 64.
[6]Bishopp, F. C., Laake, E. W., & Parman, D. C. (1926). *U.S. Dept. Agric. Farmers Bull.*, 857.
[7]Brumpt, E. (1933). *Ann. Parasit. hum. comp.*, **11**, 403.
[8]Courtis, B. (1927). *Sem. med.*, **34**, 1307.
[9]Cousin, G. (1932). *Bull. biol. France Belg. Suppl.*, **15**, 341.
[10]Davies, W. Maldwyn. (1934). *Ann. App. Biol.*, **21**, 267.
[11]Davies, W. Maldwyn, and Hobson, R. P. 1935. *Ibid.*, **22**, 279.
[12]Dove, W. E., and Parman, D. C. (1935). *Jr. Econ. Ent.*, **28**, 765.
[13]Ellis, W. (1749). A Compleat System of Experienced Improvements, etc. London.
[14]Evans, A. C. (1936). *Trans. R. Ent. Soc. London*, **85**, 363.
[15]Fitzherbarde. (1523). Boke of Husbandrie. Rycharde Pynson, London.
[16]Freney, M. R., Mackerras, I. M., and Mackerras, M. J. (1935). *J. Counc. Sci. Ind. Res. Aust.*, **8**, 161.
[17]——. (1936). *Ibid.*, **9**, 11.
[18]Froggatt, W. W. (1915). *N.S.W. Dept. Agric. Farmers Bull.*, 95.
[19]Fuller, M. E. (1934). *Counc. Sci. Ind. Res. Aust. Bull.*, 95.
[20]Gurney, W. B., and Woodhill, A. R. (1926). *N.S.W. Dept. Agric. Sci. Bull.*, 27.
[21]Hobson, R. P. (1935). *Ann. App. Biol.*, **22**, 294.
[22]——. (1936). *Ibid.*, **23**, 845.
[23]——. (1936). *Ibid.*, **23**, 852.

[24]Hogg, W. (1829). *Prize Essays, Highl. Soc. Scot. N.S.*, **1**, 325.
[25]Holdaway, F. F. (1930). *Nature*, **126**, 648.
[26]Holdaway, F. G., and Mullhearn, C. R. (1934). Counc. Sci. Ind. Res. Aust. Rep. No. 48.
[27]Hudson, H. F. (1914). *Canadian Entomologist*, **46**, 416.
[28]James, H. C. (1928). *Ann. App. Biol.*, **15**.
[29]Johnston, T. H. (1923). *Trans. & Proc. R. Soc. S. Aust.*, **47**, 201.
[30]Laake, E. W., Cushing, E. C., and Parish, H. E. (1936). *U.S. Dept. Agric. Tech. Bull.*, 500.
[31]MacDougall, R. S. (1904). *Trans. Highl. Agric. Soc. Scot.*, **16**, 128.
[32]——. (1909). *Ibid.*, **21**, 135.
[33]MacLeod, J.. Parasitology. In press.
[34]Mather, G. (1835). *Prize Essays, Highl. Soc. Scot. N.S.*, **4**.
[35]Miller, W. C. (1935). *Scot. Jr. Agric.*, **18**, 226.
[36]Patton, W. S., and Evans, A. M. (1929). Insects, Ticks, Mites and Venomous Animals. Part I. Grubb, Ltd., Croydon.
[37]Portchinsky, I. A. (1916). *Abstr. Rev. App. Ent.*, **4**, 88.
[38]Ratcliffe, F. N. (1934). *Scot. Jr. Agric.*, **17**, 249.
[39]Ratcliffe. (1935). *Ann. App. Biol.*, **22**, 742.
[40]Roth, O. (1917). *Abstr. Trop. Dis. Bull.*, **11**, 397.
[41]Salt, G. (1932). *Bull. Ent. Res.*, **23**, 235.
[42]Seddon, H. R. (1931). *Agric. Gaz. N.S.W.*, **42**, 581.
[43]Seddon, H. R., Belschner, H. G., and Mulhearn, C. R. (1931). *N.S.W. Dept Agric. Sci. Bull.*, 37.
[44]Seguy, W. (1930). *Abstr. Rev. App. Ent.*, **18**, 161.
[45]Singer, —. (1839). *Pri„e Essays, Highl. Agric. Soc. Scot., N.S.*, **6**, 132.
[46]Smit, B. (1931). 17th Rep. Dir. Vet. Ser. Anim. Indust. Un. S. Afr., 299.
[47]Stewart, N. P. (1870-71). *Trans. Highl. Soc. Scot. Ser.* 4, **3**, 260.
[48]Sutherland, W. (1893). *Trans. Highl. Agric. Soc. Scot.* 5th Ser., **5**, 80.
[49]Tusser, T. (1573). Cited by Maldwyn Davies, 1934. *Ann. App. Biol.*, **21**, 267.
[50]Wardle, R. A. (1927). *Jr. Hyg.*, **26**, 441.
[51]——. (1930). *Ann. App. Biol.*, **17**, 554.
[52]Youatt, W. (1883). Sheep : Their Breeds, Management and Diseases, New Ed. Simpkin, Marshall & Co., London.

## INFECTIOUS PLEURO-PNEUMONIA OF GOATS IN PALESTINE: COMMUNICABILITY TO SHEEP

By S. J. Gilbert, M.R.C.V.S.

Pleuro-pneumonia of goats associated with heavy mortality has been frequently observed in Palestine, more especially in severe winters. In former years the outbreaks were nearly all confined to the northern areas and practically always in the hilly districts. In these outbreaks pneumonia or pleurisy or both were stated to be a regular feature on *post-mortem* examination.

During the past two or three years a few outbreaks have been observed in the hill districts in the Jerusalem area, but have not spread to any extent, probably owing to the fact that for some years winters have been comparatively mild. The present winter, however, has been a very severe one and the deaths of large numbers of goats have been reported in the central and southern areas but not to a great extent in the northern districts, which in previous years had been chiefly affected. In the present outbreaks the veterinary officer reported that lesions of pneumonia or pleurisy did not occur in more than 20 per cent. of the field cases examined. A considerable number of sheep had also died in the same areas and with symptoms and *post-mortem* appearances resembling those seen in goats.

In 1929 a sick goat had been received at the laboratory from an outbreak typical of pleuro-pneumonia. Nasal discharge from this animal was transferred to the nostrils of a locally purchased goat, which showed rise of temperature and became emaciated. In spite of exposure in the open, neither pneumonia nor pleurisy followed and the goat recovered in the course of a few weeks. Some two years ago a similar experiment was carried out with the same results. It was observed that following such transmission, a rise of temperature took place within 48 hours.

From the present series of outbreaks, nine severely affected goats and two sheep were received. With the exception of two goats these animals all died within a few days of their arrival.

### *Symptoms in Goats.*

The most prominent symptom consists of nasal discharge which varies in amount and viscidity. According to the severity of the attack the discharge continues for two or three weeks. Pressure on the trachea easily induces a cough and some increase of lachrymal secretion is common, with subsequent caking around the eyelids. The animals become weak and emaciated and there is loss of appetite. An eruption around the lower lip is sometimes observed. The coat is harsh and dull and diarrhœa may occur. In acute outbreaks abortion is stated to have occurred in practically all pregnant infected animals.

*Post-mortem Appearances.*

In the goats examined these were not found to be striking nor were the same conditions evident in all cases. The heart muscle was usually darkened, the heart greatly dilated and the fat lying over the coronary arteries swollen and gelatinous. A small increase of pericardial fluid appeared to be usual, and sometimes there was some fluid in the pleural cavity. The liver was found to be dark and congested in all cases and the gall bladder was frequently much enlarged. Congestion of part of the small intestine was observed in three goats. In those cases which could be examined soon after death cultural examination of blood, spleen and liver proved negative. Only one goat showed typical lesions of areas of hepatisation and pleurisy. In another goat extensive pleuritic adhesions were observed and in one sheep pneumonia with extensive hepatisation.

The low incidence of lesions of pleurisy and pneumonia has been a feature of the present outbreaks, and has, therefore, led to some difficulty of diagnosis by field officers, especially in view of associated helminthiasis, and sometimes anaplasmosis. In previous outbreaks in the northern hills this difficulty has not been experienced, as lung lesions were stated to have been general, although in two experiments made they could not be reproduced under laboratory conditions.

A small portion of emulsified lung tissue from the case of pneumonia in a sheep was inoculated into a rabbit, which died on the fifth day from pasteurellosis. Following this result a rabbit inoculated from goat lung which did not show naked eye lesions died within 48 hours. This was again positive in two other cases in which there were no lesions of pneumonia, but negative in others. Further, the result could not be repeated in a number of cases from lung tissue of both sheep and goats forwarded from the field. Lung material taken from two normal goats killed at the local slaughterhouse were negative to rabbit inoculation.

A sheep inoculated intratracheally with 1 c.c. of *Pasteurella* culture from the first rabbit showed fever for some days (highest temperature 106° F.). A goat was similarly inoculated with a culture of pasteurella isolated through a rabbit from goat lung and also received an intra-nasal injection of bacteria-free filtrate of nasal discharge from a sick goat. This goat passed through a moderate attack of the disease. A further goat (No. 1) was injected intra-nasally with filtrate only and passed through a very severe attack (highest temperature 105° F.). Continuous diarrhœa, emaciation, and discharge from the nose and eyes occurred, and subsequently a herpes-like eruption on the outer side of the lower lip. Slow recovery took place after some three weeks. The filtrate was obtained by washing out nasal discharge with saline and passing through a Seitz filter. It was kept in cold storage for 24 hours before use.

A goat (No. 5) was inoculated intravenously with filtrate of nasal discharge from a naturally infected goat. This was followed by temperature reactions. The nostrils were dry and there was some lachrymation and coughing.

A very noticeable feature in all cases of infection is the variation of temperature from day to day. In naturally infected animals temperatures fall before death to sub-normal, descending to 98° F. or lower. The following are the temperature records showing the course of fever in experimentally infected animals and one natural case of infection.

| *Goat No.* 1. | | *No.* 2. | | *No.* 5. | | *No.* 3. | |
|---|---|---|---|---|---|---|---|
| | | *Infected with* | | | | | |
| *Filtrate Intra-nasally.* | | *Transfer of Nasal Discharge.* | | 1 *c.c. of Filtrate of Nasal Discharge Inoc. Intravenously.* | | *Natural Infection.* | |
| *Morning.* | *Evening.* | *Morning.* | *Evening.* | *Morning.* | *Evening.* | *Morning.* | *Evening.* |
| 102·2 | 104 | 101·4 | 102·6 | 104 | 104·2 | 102·2 | 103·6 |
| 103 | 105 | 101 | 103 | 103·2 | 104 | 102·8 | 103·6 |
| 103 | 103·4 | 103·2 | 103 | 103·6 | 104·4 | 102·6 | 103·4 |
| 104 | — | 102·4 | 103 | 103·6 | 104·2 | 102·4 | 103·6 |
| 105 | 105 | 102·6 | 102·8 | 102·8 | 104·6 | 104 | 104 |
| 103·4 | 105·4 | 103·2 | 104 | 103 | 104·6 | 104·4 | 104·8 |
| 105·6 | 105 | 103·2 | 103 | 102·8 | 104 | 104·8 | 105 |
| 104·2 | 104 | 103 | 103·2 | 102·2 | 103·4 | 102·8 | 103·4 |
| 105·4 | 105·6 | 103·2 | 104 | 102·8 | 103·8 | 104·2 | 104·4 |
| 101·6 | 102·4 | 101·6 | 103 | 102·2 | 103·2 | 103·4 | 103·2 |
| 98·4 | — | 102·2 | 102·4 | 101·6 | 103 | 101·4 | 103·2 |
| 100·4 | 102 | | | 101·8 | 103·8 | 102 | 103 |
| 101·4 | 103 | | | 102·8 | 103·8 | 99·4 | 104 |
| 102·6 | 103·8 | | | 101·8 | 103·8 | 100·6 | 104 |
| 102·4 | — | | | 102 | 103·6 | 102 | 101·2 |
| 102·6 | 103·8 | | | 102 | — | 101·8 | 102 |
| 102·4 | 103·2 | | | | | 102·2 | 103·2 |
| 102 | 103 | | | | | 101·4 | 103·8 |
| 102·4 | 103·2 | | | | | 102·8 | 103 |

Goat No. 3 was a field case already severely infected. This animal survived. Goat No. 2 was only slightly affected clinically.

No goats infected experimentally and maintained at the laboratory stable have died, even though continuously exposed in the open. Such conditions, however, do not compare with those occurring in the hill villages. Sick goats received from the field were already severely affected and most died within a few days of arrival. Exposure to severe winters in the hills and lack of proper feeding are mostly responsible for the severity of the disease.

*Associated Conditions.*

(*a*) Helminthiasis.

Village goats and sheep are generally infested with worms. Mortality associated with severe stomach worm infestation is

of annual occurrence. This varies in severity from year to year and is usually more marked in August and September. In the present cases some goats showed great numbers of worms in the bronchi and intestines, but very severe infestation was not found in all cases.

(*b*) Anaplasmosis.

Native sheep and goats exposed to tick infection are practically all infected with anaplasms. These do not appear to do a great deal of harm under normal conditions when the animals are properly fed and stabled. In conditions of starvation and exposure in the winter, breakdowns occur and have been associated with severe mortality. Blood examination reveals the presence of anaplasms and severe blood changes. The visible mucous membranes are usually icteric, the disease being locally known as " safra " or yellows. On *post-mortem* examination a yellowness of the skin and organs is apparent. Spleen and lymphatic glands are enlarged. A high rate of abortion has also been frequently observed in connection with anaplasmosis.

Blood examinations in the present series did not show blood changes and in only one or two cases were anaplasms observed.

(*c*) Pasteurellosis.

As previously stated, *Pasteurella* virulent for rabbits have been found in the lungs of both sheep and goats in the present outbreaks. Rabbit inoculation of lung material, however, was positive only in a minority of cases and all cultures of blood and organ material taken soon after death were negative.

(*d*) *Hæmophilus*.

Attempts to isolate a *Hæmophilus* from the bronchi on two occasions have been negative.

*Communicability to Sheep.*

During the same period in which goats have been dying in large numbers a heavy mortality in sheep has also occurred. Thus, during the month of November, 1936, the returns submitted by field officers show that 13,220 goats and 417 sheep had died. The returns for December report the deaths of 5,454 goats and 2,316 sheep. Enquiry from individual officers elicited an estimated infection of 60 to 80 per cent. in goats and 40 to 50 per cent. in sheep. Of infected animals mortality is estimated at 40 to 60 per cent. in goats and 25 to 35 per cent. in sheep. The higher percentages apply to the more exposed and hilly areas.

Two sick sheep were received and showed symptoms identical with those which were seen in goats, nasal discharge, emaciation, etc. These sheep died and the *post-mortem* appearances closely resembled those observed in goats. Organs submitted from field cases also showed these appearances.

The following experiments were later carried out on locally purchased sheep :—

(1) Transfer of nasal discharge from a naturally infected goat to the nostrils of sheep No. 27.

A slight increased discharge from the eyes and nostrils followed. Temperature slightly above normal.

(2) Filtrate of nasal discharge from a naturally infected goat injected intra-nasally in sheep Nos. 40 and 42.

A very definite discharge, slight lachrymation, and cough followed. Temperatures were higher than in previous experiment. Sheep No. 40 produced a premature lamb on the 5th day and the lamb died next day.

(3) 1 c.c. filtrate of nasal discharge from naturally infected goats inoculated intravenously into sheep No. 43.

Very profuse and thick nasal discharge. Temperatures generally higher than in experiments 1 and 2.

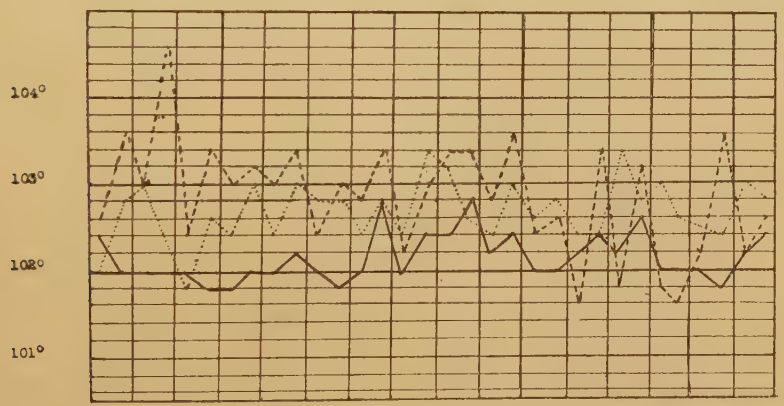
(4) Nasal discharge from sheep No. 40 washed out with saline and injected into the nostrils of goat No. 6.

Variation of daily temperature but not above 103·8° F. The nostrils were very dry and there was continuous coughing.

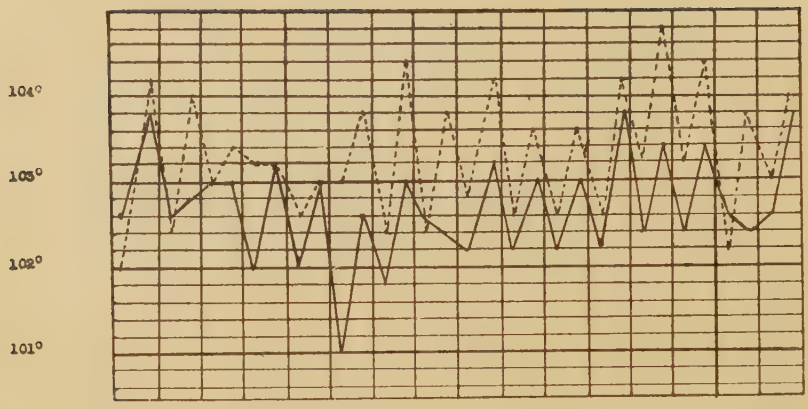
(5) Filtrate of nasal discharges of sheep Nos. 40 and 42 taken on the eighth day and injected intravenously into goat No. 7.

This animal showed diarrhœa but no nasal discharge. Temperature reactions very slight.

Apart from the field evidence, the experimental findings indicate that the disease is communicable to sheep. Experimentally they are much less susceptible than goats, and this appears to be also the case in the actual outbreaks. In the case of experimental infection they are not so severely affected and recover more quickly.




- - - - - - Sheep No. 42.* Intranasal injection of filtrate of nasal discharge from goat (natural infection).
. . . . . Sheep No. 27.* Transfer of nasal discharge from goat (natural infection).
———— Normal sheep No. 35.
* Morning and evening temperatures from date of inoculation.




- - - - - - Sheep No. 43.* Intravenous injection of filtrate of nasal discharge from goat (natural infection).
———— Goat No. 6.* Intra-nasal injection of nasal discharge from sheep No. 40.
* Morning and evening temperatures from date of inoculation.

## Conclusions

(1) Investigation of an outbreak of infectious pleuro-pneumonia of goats in Palestine is described.

(2) The incubation period is less than 48 hours.

(3) The disease is caused by a filterable virus.

(4) The virus is shown to be communicable to sheep. Sheep are less susceptible than goats.

(5) The disease is not likely to be fatal except when the animals are exposed to severely cold weather and are in low condition.

# A PRELIMINARY REPORT ON THE PROBLEM OF THE BOVINE SKIN-LESION TUBERCULIN-REACTOR

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## Introduction.

In recent years extensive schemes for the eradication of bovine tuberculosis have been in progress in the United States of America and in Canada. Veterinarians in these countries have found that as the incidence of the disease was reduced their work was increasingly complicated by the occurrence of "no-visible-lesion reactors" and the so-called "skin-lesion reactors." The recent increased attention to eradication in this country has resulted in the recognition of similar "skin-lesion reactors" in herds which are considered free of tuberculosis. This article is a description of twelve cases that have come to our notice, our experiments with the material obtained and a very brief description of the histology of the lesions. We intend to include in a later publication a more detailed account of the histology and a comparison of the naturally occurring lesions with those artificially produced by various known types of acid-fast organisms.

There seems to be no reason to doubt that these lesions may frequently be present in bovine animals, but little attention was paid to them until they were found in herds considered tuberculosis free, where they have apparently produced a varying degree of sensitisation to the intradermal tuberculin test. Such animals on *post-mortem* examination show no systemic lesions of tuberculosis, even on intensive search coupled with biological examination of the lymphatic glands. When the skin and subcutaneous tissues are closely examined a varying number of nodules are found in and under the skin which frequently contain acid-fast organisms and have a histological structure closely resembling that of a tubercle.

These lesions occur mainly on the legs, though occasionally they may be found on the shoulder, the flank or (rarely) the back. In a few cases they have been reported as occurring on the teats or the udder. They usually take the form of nodules in the subcutaneous tissues but may involve or even be confined to the skin itself. Frequently several nodules are present, definitely separated from one another and varying in size from a small pea to a hen's egg. Multiple lesions are occasionally found, forming a chain along the lymphatics, which may be thickened and enlarged. There are no clinical signs of inflammation, the lesions being firm, cold and non-painful. The larger nodules are readily detected on running the hand over the affected region, but smaller lesions may escape detection until the carcase is skinned. In a number of cases there are

abrasions in the skin over the nodules and sometimes there is definite ulceration at the surface. Where only the skin is involved all that may be seen is a hairless patch, which can easily be missed if overlapped by the surrounding hair. Lesions have also been recorded by other workers as occurring on the teats in the form of small, firm nodules involving the skin, skin ulcers or the scars of healed ulcers.

Section of the subcutaneous nodules usually shows a brownish lesion with a fibrous matrix and small yellowish granules throughout. Some cases show pockets of yellowish pus enclosed in fibrous capsules. Lesions confined to the skin consist of a brownish area in the dermis corresponding to a hairless patch or an ulcer on the outer surface.

In smears made from the lesions varying numbers of short acid-alcohol-fast organisms can generally be found. Histologically the lesions have the structure of a granuloma, *viz.*, a small, round-cell infiltration, epithelioid, plasma and giant-cells, with necrosis and sometimes caseation and even calcification. Attempts to identify the acid-fast organisms by cultural methods or small-animal inoculation have so far been unsuccessful.

## Literature.

The first recorded observation of these so-called skin-tuberculosis lesions appears to be that of Perard and Ramon in 1913[1]. These observers compared the lesions with the tuberculides of human pathology and considered they were dealing with *M. tuberculosis* of weakened virulence.

In 1916, Traum[2] recorded six cases of " lymphangitis " in cattle from two tubercle-free herds. The lesions were suggestive of Bovine Farcy (streptothricosis), but microscopic examination showed the presence in the lesions of acid-alcohol-fast organisms somewhat similar to tubercle bacilli. Tested intradermally with avian tuberculin, four out of six animals gave reactions, but no reactions were obtained with bovine tuberculin. Inoculation of various culture media and of small laboratory animals proved negative and Traum concluded that he was not dealing with mammalian tuberculosis.

In 1919, he made a further report[3] on seven cases and in 1923 on 25 more cases[4]. All these were reactors to the intradermal tuberculin test in which no other lesions could be found. Animal inoculations were again negative but in one case he isolated a rapidly growing chromogenic acid-fast organism which gave rise to abscess formation in experimental animals. He hesitated, however, to incriminate it as the cause of the disease described. In a further report[5] he recorded failure to infect guinea-pigs, rabbits or chickens with tuberculosis by using large doses of culture, and calves injected intravenously and subcutaneously showed no result after a year although a transient swelling followed subcutaneous injection.

Wright[6] described eight skin cases from 100 tuberculin reactors. Four of these had other lesions of tuberculosis as well. Day[7] found 47 skin cases in 85 tuberculin-reactors autopsied. Attempts to infect

chickens by feeding with skin-lesion material over a considerable period of time failed[8]. Later[9], he reported on 200 cases examined. Cultural examination proved negative, but by keeping inoculated guinea-pigs five to six months he was able to obtain typical lesions of tuberculosis. He also claimed the successful transmission of the disease to one calf out of six, using skin-lesion material. He concluded that skin-lesions were due to *M. tuberculosis* which had gained entrance to the skin by wound contamination from infected manure.

Carpenter and Goldberg[10] studied 30 cases but failed completely in attempts to infect small animals. Histological comparison of the lesions with a number of cases of lupus in man showed great similarity between the two and they considered the skin lesions to be due to tubercle bacilli of weakened virulence.

Marsh[11] studied 60 tuberculin-reacting cows which showed skin-lesions on the teats and in which no other lesions of tuberculosis could be found ; 75 per cent. of the cases showed acid-fast bacilli in smears and 36 cases were used for cultural examination and small-animal inoculation. Negative results were obtained though the animals were kept for periods up to five months. Later[12], 38 other cases were studied with similar results.

Crawford[13], reviewing the literature previous to 1926, quotes the evidence in support of the possibility that the lesions are in some instances caused by acid-fast organisms other than the tubercle bacillus. Later[14], after getting entirely negative results with 20 skin cases, he attempted to reproduce the skin-lesions by inoculating the abraded skin of cattle with bovine, avian and human tubercle bacilli and three strains of saprophytic acid-fast organisms. Intradermal and subcutaneous inoculations were also made. The five last-named organisms failed to produce lesions of any sort. In the case of the bovine tubercle bacillus no skin-lesions were obtained, but the local lymphatic glands became affected. All the animals were tuberculin-tested intradermally four months after inoculation, and only that inoculated with the bovine tubercle bacillus gave a reaction.

Crawford's results are in contrast with those of Carpenter and Goldberg[10], who claimed to have produced subcutaneous lesions closely simulating skin-lesions by inoculation of a culture of bovine tubercle bacilli. It is noteworthy that in their case the skin passage did not result in loss of virulence of the organisms.

Mitchell[15] records collected data with reference to 133 cases examined. Extensive attempts at cultivation resulted in many different organisms being obtained but none were acid-fast. Two types were fairly consistently found but these proved avirulent for experimental animals. Animal inoculation was tried with calves, guinea-pigs, rabbits and chickens, and after approximately 100 attempts with material from 33 cattle he obtained lesions of tuberculosis in one rabbit, which died 131 days after inoculation. (Unfor-

tunately there was no control to this, as the only other animal inoculated with similar material was a guinea-pig which died of pneumonia 18 days after inoculation.) Mitchell looks on the lesions as truly tuberculous but possibly caused by a strain somewhat atypical in its selection of unusual tissue for colonisation.

Graham *et al*[16] record negative findings in two cases studied.

Feldman[17] tried to incite lesions in laboratory animals by intracerebral injection of emulsions prepared from 14 typical subcutaneous nodules. No macroscopic lesions were produced, but definite collections of epithelioid cells somewhat similar to tubercles were observed in the brains of the rabbits used. Van Es[18] examined 40 different sets of " tuberculous " lesions of the skin and subcutis. Acid-fast bacilli were found in 36 cases. All the typing experiments undertaken yielded negative results. Tuberculosis-free pigs were fed with considerable amounts of bovine skin-lesion material but when tested some months later with avian and mammalian tuberculin they were negative, and no tuberculous lesions were found at autopsy.

Daines and Austin[19, 20], studied the subcutaneous lesions in 225 cattle, and by making cultures on a modified Petroff's medium they obtained various organisms which fell into four main groups :—

(1) A typical virulent bovine strain of *M. tuberculosis* obtained in one case only. (This animal had visceral lesions of tuberculosis as well.)

(2) A partially acid-fast, Gram-positive, pleomorphic, diphtheroid organism, producing in 12 to 24 hours colonies which were usually chromogenic.

(3) A slow-growing, acid-fast, Gram-positive, diphtheroid rod-shaped organism with occasional coccoid forms, producing deep orange colonies in about seven weeks.

(4) A non-acid-fast, Gram-positive, pleomorphic organism occurring as long or short rods or coccoid forms. This produced smooth, non-chromogenic, spreading colonies in 24 hours.

The majority of their cultures belonged to group (2) and showed a close similarity to the Preisz-Nocard bacillus. Small animals inoculated with groups (2), (3) and (4) failed to develop lesions of tuberculosis. Subcutaneous injection of cattle with the group (3) organism evoked the formation of nodules in the subcutaneous tissues which remained up to about four months and one animal developed a transient sensitisation to tuberculin. In a further article[21] they claimed that fairly characteristic skin-lesions had been produced experimentally by injecting these acid-fast strains into rats, white mice, guinea-pigs and cattle, but their evidence is unsatisfactory. Later[22] they describe a simpler and quicker method of isolating their group (3) organism in fresh uncooked egg-yolk.

In the reports[23] and [24] of the Chief of the Bureau of Animal Industry, U.S.A., for 1932 and 1933 mention is made of two strains of acid-fast organisms recovered from skin-lesions, which caused transient tuberculin sensitisation in cattle. No details of these

cultures are given. In 1934, it is reported[25] that four cattle inoculated with these organisms developed swellings at the site of inoculation which in the case of two animals gradually subsided, but in the other two became very pronounced and were still visible eight months after inoculation. None of the cattle showed a sensitisation to mammalian tuberculin up to the seventh month, but all reacted positively to avian tuberculin at the end of the third month. From one of these cattle, killed at the end of the eighth month, a skin-lesion was removed from which an acid-fast organism, apparently identical with that injected, was recovered in pure culture.

Feldman[26] reports work with two strains of acid-fast organisms obtained from Daines and Austin, representing their groups (2) and (3) respectively. Tubercle-free fowls were inoculated by various routes, but although they were tested over a period of six months none developed any reaction to avian or mammalian tuberculin. No details of the *post-mortem* examinations are given. Later[27] the same worker injected these organisms intraperitoneally into white mice but they had no pathogenic effect, being in marked contrast to avian, bovine and human strains of *M. tuberculosis* which were also used. He concluded that these skin-lesion strains are more allied to the saprophytic than to the pathogenic acid-fasts.

Feldman and Schlotthauer[28] record the examination of eleven reacting cattle obtained at various tests from a " tubercle-free " herd. Three of these proved to be cases of so-called " skin tuberculosis." In two of these cases they claimed to have isolated strains of the avian tubercle bacillus from the mesenteric glands, while findings in the third case were entirely negative.

According to Prendergast[29] 95 per cent. of several hundred skin-lesion reactors examined failed to show internal lesions of tuberculosis on *post-mortem* examination. Many of these cases were from herds which had passed several tests with no reactors.

Crawford[30] records the isolation of two apparently identical acid-fast organisms recovered from skin cases from widely separated districts. The organisms were isolated by culture from the apparently normal spleens of guinea-pigs that had been injected with skin-lesion material. Attempts to isolate similar organisms from normal guinea-pigs were unsuccessful. Cultures of these organisms proved non-pathogenic for guinea-pigs, rabbits and fowls, but when injected subcutaneously into cattle they produced lesions " somewhat similar " to skin lesions. Some of the cattle developed a suspicious reaction to mammalian and were positive to avian tuberculin. The organisms were recovered in pure culture from the lesions.

A " tuberculin " was prepared from these organisms and used in field tests of herds in the hope that it might prove useful in differentiating " skin tuberculosis " from cases of true tuberculosis. It was found, however, that standard tuberculin was a better agent for eliciting reactions, both in animals which were definitely affected with tuberculosis and in those only harbouring skin-lesions.

Crawford hesitates to incriminate these organisms as being the causal agent of the bovine skin-lesions, but concludes that the most logical explanation of the latter is that they are the result of infection with soil acid-fast organisms and lead to a " group " rather than a " specific " sensitisation to tuberculin.

### Cases Recorded at the Veterinary Laboratory, Weybridge.

During the past two years several cases of "skin tuberculosis" have been encountered in cattle which, in connection with the administration of the Attested Herds Scheme, showed inconsistent or inconclusive reactions to the intradermal tuberculin test. Forty-eight animals of this kind have so far been examined and, of these, twelve have shown skin-lesions. The first four cases described were dealt with by A. V. Franklin, M.R.C.V.S., and J. Reid, M.R.C.V.S., and the remainder by the writers.

*Case* 1. (Cow 733.) The first case recorded at this Laboratory was reported by Franklin on 25.3.35[31]. The animal had given a doubtful reaction to the intradermal tuberculin test (Table I). The only lesion found at *post-mortem* was under the skin on the outer aspect of and just below the left elbow-joint. It consisted of a series of intimately connected pea-size nodules in the subcutaneous tissue, the whole being about three inches in length. Each nodule contained thick, yellow, glairy pus enclosed in a fibrous capsule. Smears made from the pus showed acid-fast organisms. The histological structure of the nodules was similar to that of tuberculosis. Six guinea-pigs, six rabbits and three tuberculin-tested fowls were inoculated with material from the lesions. These were killed two months later but none showed any lesions of tuberculosis.

*Case* 2. (Cow 747.) (A. V. Franklin[31].) This animal had originally given a positive reaction to an intradermal test with bovine tuberculin. When retested six weeks later it gave a doubtful reaction to bovine tuberculin and a positive reaction to avian tuberculin. No reaction was elicited by the injection of synthetic medium, used as a control (Table I). At *post-mortem* the only lesion found was a nodule, about the size of a filbert nut, situated under the skin about six inches above the hock on the outside of the left hind leg. It lay in the subcutaneous tissues, was firm in consistence and was composed of a fibrous wall enclosing blood-stained pus. Acid-fast organisms were present in smears. Eight guinea-pigs, three rabbits and three fowls were inoculated with material from the lesion, but all proved to be negative on *post-mortem* examination three months later. The fowls used had been tuberculin-tested prior to inoculation and were negative. They were retested with negative results immediately before being killed.

*Case* 3. (Cow 748.) (A. V. Franklin and J. Reid[31].) This animal, which had given a positive tuberculin test on 12.4.35, proved negative to both bovine and avian tuberculins on 28.5.36. It was tested on two subsequent occasions with both bovine and avian tuberculin, with a three months interval between the tests. A doubtful reaction was obtained with bovine tuberculin at the last test; the remainder were negative (Table I). At the *post-mortem* the only lesions found were a skin-lesion about the size of a walnut just above the lateral aspect of the left hock and several smaller adjacent lesions. The cut surfaces of the lesions were fibrous, with small

encapsulated pockets of pus. A few short, thick acid-alcohol-fast bacilli were found in smears. Material was inoculated into 14 guinea-pigs, six rabbits, three tuberculin-tested fowls, three pigeons and three white mice, by subcutaneous, intraperitoneal, intravenous or intradermal routes. Inoculations were also carried out with emulsions from the various lymph glands. Cultures direct and after antiformin treatment all proved negative. The experimental animals were killed two months after inoculation but no evidence of tuberculosis could be found.

*Case* 4. (Cow 767.) (J. Reid[31].) This animal had given a positive tuberculin reaction on 20.1.36, but when retested at the Laboratory on 3.2.36 it gave doubtful reactions to both bovine and avian tuberculins (Table I). A small subcutaneous nodule was found over the right carpus which resembled previous skin lesions, but no acid-fast organisms were found in smears. Guinea-pigs, rabbits and fowls were inoculated with material from the lesions and also from the lymphatic glands. Two months later they were killed but showed no signs of tuberculosis.

*Case* 5. (Cow 793.) This animal, which was five years old, had been tuberculin-tested with negative results at six-monthly intervals from 1932 till April, 1936, when it gave a doubtful reaction ; tested a week later with avian tuberculin it gave a marked reaction (Table I). Apart from a tumour on the left shoulder, the only lesions found at autopsy were in the skin. There was one swelling about the size of a hazel nut behind the left metacarpus, and three small pea-size nodules beside it. The larger nodule was entirely subcutaneous and on section was found to consist of dense brownish tissue with small necrotic nodules scattered throughout. A few acid-fast organisms were found in smears. The smaller nodules were largely intradermal in position and communicated with the surface, where loss of hair or a hæmorrhagic crust occurred. Acid-fast organisms were also present in these intradermal lesions. Two other lesions were found, both entirely subcutaneous and about the size of brazil nuts ; one was situated on the lateral aspect of the left carpus and the other just above the right shoulder joint. A few acid-fast organisms were detected in both. Cultures were made, both direct and after antiformin treatment, on various media—Dorset's egg, glycerine egg, serum agar, glycerine-serum agar, blood agar, glycerine agar, glycerine potato, Löwenstein-Jensen medium and Twort's medium for Johne's bacillus. We could detect no evidence of growth five months after inoculation, and smears then made showed no acid-fast organisms. Eight guinea-pigs, four rabbits and four fowls were inoculated by s/c, i/p and i/v routes with material from the lesions, and a number were inoculated with emulsions prepared from the main lymphatic glands. None of these had developed any lesions of tuberculosis when examined *post-mortem* two months later.

*Case* 6. (Cow 803.) Born in March, 1933, and tuberculin-tested at six-monthly intervals. In May, 1936, a suspicious reaction was obtained. When retested after a few weeks it was positive. Three weeks later it was tested at the Laboratory with bovine and avian tuberculins, and reacted to both (Table I).

A large number of skin lesions were present in the form of small scars scattered over the outer surface of the fore limbs. They extended from the shoulder to the metacarpus, but were mainly on the forearm. On section these proved to be small, firm, brownish nodules in the substance of the dermis, with scars on the surface. Smears from these lesions showed the

presence of a few acid-fast bacilli. Cultures were made on various media as before, and experimental animals and fowls were inoculated with material from the lesions. No macroscopic lesions were found in any of the lymphatic glands, viscera or other organs. Small animals and tuberculin-tested fowls were inoculated from the glands.

The cultures were discarded as negative after three months, and the inoculated animals again failed to develop lesions, although they were not killed till three months after inoculation.

*Case* 7. (Cow 804.) A first-calf heifer, about three years old, which was negative to the tuberculin test until May, 1936, when the first positive reaction was obtained. A week later this was confirmed by a second test. A further test at the Laboratory three weeks later gave a negative result with bovine tuberculin but a positive one with avian (Table I). A skin-lesion about the size of a hazel nut was situated on the lower part of the left flank, and a few small lesions occurred on the limbs. Smears showed fairly plentiful acid-fasts, but cultural methods and small-animal inoculation failed to isolate any acid-fast organisms. No other macroscopic lesions were found and biological examination of the lymphatic glands proved negative.

*Case* 8. (Cow 808.) This animal had given a doubtful reaction to bovine tuberculin on July 3rd and again on July 16th, 1936 (Table I). The only *post-mortem* lesion was a subcutaneous nodule, about the size of a brazil nut, in the neighbourhood of the right shoulder joint. It was similar on section to those previously described, and a few acid-fast organisms were found in smears. The inoculation of small animals and fowls with material from the lesion and from the lymphatic glands proved negative. A three-months-old calf, which had passed several tuberculin tests, was inoculated subcutaneously in a fore limb with an emulsion prepared from the lesion. No swelling developed and the calf gave no reaction to avian or bovine tuberculin six months later.

*Case* 9. (Cow 809.) This animal had passed ten tuberculin tests prior to May, 1936, but gave doubtful reactions to bovine tuberculin on July 3rd and July 16th, 1936 (Table I). A small lesion was found on the back, consisting of a hairless patch about the size of a shilling. The lesion was limited to the dermis and scrapings showed acid-fast organisms to be very plentiful. Cultures were made on various media and the remainder of the lesion was ground up and injected subcutaneously into a tuberculin-tested calf. No lesion developed at the site of inoculation and the calf was still negative to bovine tuberculin, and also to avian tuberculin and johnin, six months after injection. Cultures were still negative after four months incubation.

*Case* 10. (Heifer 815.) This animal gave a negative reaction to bovine tuberculin from August, 1934, until May, 1936. On May 1st it gave a doubtful reaction, but when retested a week later it was negative. Further tests with bovine tuberculin on July 21st and August 6th both elicited doubtful reactions, and a negative reaction was obtained with avian tuberculin on the latter date (Table I). The only lesions found were in the skin and subcutaneous tissues of the lower parts of the limbs. All four limbs were affected and seven separate lesions were found, varying in size from a pea to a large bean. One of the lesions showed small pockets of yellowish pus but the remainder had the usual brownish fibro-granular appearance. Two of the lesions were confined to the dermis and corresponded to hairless patches on the outer skin surface. Smears from all the lesions showed the presence of acid-fast organisms in varying numbers.

Skin-lesion material was employed to inoculate cultures on various media and was also inoculated into small animals and fowls. A tuberculin-tested calf was given subcutaneous and intravenous injections with similar material. The experimental animals and fowls showed no signs of tuberculosis when killed three months later, and attempts at cultural isolation also failed. The usual small-animal inoculations with material from the lymphatic glands were also negative. The calf developed no local lesion and failed to react to either bovine or avian tuberculin or johnin four months after injection.

*Case* 11. (Cow 816.) After giving a doubtful reaction to bovine tuberculin on July 23rd, 1936, this animal was retested at the Laboratory with bovine and avian tuberculins on August 6th. It proved positive to bovine and negative to avian tuberculin (Table I). Retested with dilute bovine tuberculin by the subcutaneous route five weeks later, it again gave a positive reaction (Table II). At *post-mortem* examination we were unable to detect any macroscopic lesions of tuberculosis in the lymphatic glands, organs and viscera. Biological examination of the glands for tuberculosis proved negative. A number of extensive lesions were found in the subcutaneous tissues of all four limbs. These took the form of fibrous nodules containing pockets of viscid yellow pus. They occurred in rows and were connected by enlarged and thickened lymphatics. Smears showed a few acid-fast organisms to be present, mainly in the more fibrous portions of the lesions. Animal inoculation and cultural experiments with these lesions proved negative.

*Case* 12. (Heifer 817.) When tested with bovine tuberculin on 21.7.36, this animal gave a doubtful reaction. Retested at the Laboratory on 6.8.36 it was positive to bovine tuberculin and negative to avian (Table I). A subcutaneous tuberculin test applied five weeks later gave a positive result. No lesions of tuberculosis were observed on *post-mortem* and the routine animal inoculations with gland material proved negative. There were extensive lesions in the subcutaneous tissues of the limbs, very similar to those found in Case 11. A few of the lesions involved the dermis. Acid-fast bacilli were found in smears but were scanty. Material from these skin lesions failed to yield any positive results when used for animal or culture inoculations.

## Histological Structure.

The structure of the skin lesions we have examined is, in general, like that of tuberculous granulomata ; necrosis, caseation, and sometimes calcification may be seen in addition to the characteristic epithelioid cell and lymphocyte invasion. Giant-cell formation is very frequent. Lesions from different animals and different lesions from the same animal, show a considerable diversity of structure. In lesions of the dermis there is no zonal or regular arrangement, the granulomatous tissue appearing to invade irregularly and to replace the hair follicles and elastic tissue with epithelioid and lymphocytic cells. The hair follicles appear resistant, since easily identifiable remnants occur in the middle of foci of granulomatous tissue. In other instances the lesions appear to originate round a follicle root, which persists with the hair bulb invaded and the papillæ often necrotic. Unless ulceration occurs the epidermis shows no change, except possibly a thickening and a deepening of the

INTRADERMAL TUBERCULIN TESTS OF SKIN-LESION REACTORS.

S.O. = Slight œdema ; O. = œdema ; D.O.= Diffuse œdema ; H. = Heat ; P. = Pain.

| *Number of Animal.* | *Date of Test.* | *Tuberculin Used.* | *Skin Measurement—mm.* | | | *Description of Reaction. 72 hours.* | *Caudal Fold.* | *Result.* |
|---|---|---|---|---|---|---|---|---|
| | | | *Before Injection.* | *48 hours.* | *72 hours.* | | | |
| 733 | Not recorded | Synthetic Bovine | 6 | 10 | 10 | O. | — | Doubtful |
| 747 | 12.4.35 | S. Bovine | 6 | 8 | 12 | D.O. | + | Positive |
| | 28.5.35 | S. Bovine | 8 | 9 | 11 | S.O. | Slight swelling | Doubtful |
| | | S. Avian | 8 | 14 | 14 | D.O. | | Positive |
| | | Control | 8 | 8 | 8 | | | |
| 748 | 12.4.35 | S. Bovine | 6 | 11 | 18 | D.O. | + | Positive |
| | 28.5.35 | S. Bovine | 7 | 9 | 11 | Firm swelling | — | Negative |
| | | S. Avian | 7 | 12 | 12 | S.O. | | Negative |
| | | Control | 7 | 7 | 7 | | | |
| | 10.7.35 | S. Bovine | 8 | 10 | 14 | Firm swelling | — | Negative |
| | | S. Avian | 8 | 8 | 12 | | — | Negative |
| | 2.10.35 | S. Bovine | 8 | 10 | 16 | Firm and circumscribed | — | Doubtful |
| | | S. Avian | 8 | 10 | 10 | | — | Negative |
| 767 | 20.1.36 | S. Bovine | 4 | 6 | 12 | S.O.<br>P. | — | Positive |
| | 3.2.36 | S. Bovine | 6 | 8 | 12 | S.O. | — | Doubtful |
| | | S. Avian | 6 | 10 | 12 | Firm swelling | — | Doubtful |
| | | Control | ——— No Reaction ——— | | | | | |
| 793 | 27.4.36 | S. Bovine | 7 | 10 | 10 | S.O. | Slight swelling | Doubtful |
| | 4.5.36 | S. Avian | 8 | 20 | 27 | D.O.<br>P. | + | Positive |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 803 | 11.5.36 | S. Bovine | 8 | 14 | 15 | Firm and circumscribed | Slight swelling | Doubtful |
| | 19.5.36 | S. Bovine | 6 | 10 | 14 | D.O. | — | Positive |
| | 6.6.36 | S. Bovine | 7 | 12 | 15 | D.O. P. | — | Positive |
| | | S. Avian | 6 | 15 | 18 | D.O. P. | + | Positive |
| 804 | 11.5.36 | S. Bovine | 7 | 14 | 14 | Firm swelling | + | Positive |
| | 19.5.36 | S. Bovine | 7 | 8 | 12 | S.O. | + | Positive |
| | 11.6.36 | S. Bovine | 7 | 9 | 10 | | — | Negative |
| | | S. Avian | 7 | 12 | 14 | S.O. | + | Positive |
| | | Control | 7 | 8 | 7 | | | |
| 808 | 3.7.36 | S. Bovine | 6 | 8 | 15 | S.O. | — | Doubtful |
| | 16.7.36 | S. Bovine | 6 | 8 | 11 | S.O. | — | Doubtful |
| 809 | 3.7.36 | S. Bovine | 6 | 8 | 10 | S.O. | — | Doubtful |
| | 16.7.36 | S. Bovine | 6 | 8 | 9 | S.O. | — | Doubtful |
| 815 | 15.5.36 | S. Bovine | 7 | 11 | 12 | Firm nodule | Slight swelling | Doubtful |
| | 22.5.36 | S. Bovine | 8 | 10 | 11 | Firm pea | — | Negative |
| | 21.7.36 | S. Bovine | 8 | 8 | 12 | Firm nodule | Slight swelling | Doubtful |
| | 6.8.36 | S. Bovine | 10 | 10 | 13 | S.O. | — | Doubtful |
| | | S. Avian | 9 | 9 | 10 | | — | Negative |
| 816 | 23.7.36 | S. Bovine | 7 | 9 | 16 | Firm swelling | — | Doubtful |
| | 6.8.36 | S. Bovine | 6 | 10 | 14 | D.O. | + | Positive |
| | | S. Avian | 6 | 9 | 10 | Firm pea | — | Negative |
| 817 | 21.7.36 | S. Bovine | 8 | 10 | 12 | | Slight swelling | Doubtful |
| | 6.8.36 | S. Bovine | 8 | 11 | 12 | S.O. | + | Positive |
| | | S. Avian | 8 | 9 | 9 | | | Negative |

papillæ of the rete mucosum; with ulceration the epidermis disappears and the granulomatous tissue becomes exposed at the surface. Subcutaneous lesions, or lesions in the deeper parts of the dermis, sometimes show well-marked lobules separated by a variable amount of newly-formed connective tissue, while sometimes there is no lobulation and very little connective tissue. In one case, where pus had been noted macroscopically, examination of the section showed the new tissue arranged in a ring round a cavity which was partially filled by necrotic and keratinous-looking material and lined in part by stratified epithelium, suggesting either some invagination of epidermis and stratum corneum, or a diseased and expanded hair follicle. Leucocytes, particularly those with a lobed nucleus and acidophile granules, are plentiful in all sections. Plasma cells also are prominent, frequently occurring in little nests as well as being more sparsely distributed. The lesions are all very vascular, and in some cases moderately severe hæmorrhage has occurred in the lesion, especially at the margin of the lesion and at the margin of the necrotic zones.

To-day tuberculous lesions of the skin of cattle are infrequently encountered, and before drawing any conclusions we intend to examine the histological effects of inoculating cattle intradermally with known strains of *M. tuberculosis*.

## Discussion.

Thorough *post-mortem* examinations of our twelve cases have revealed no lesions other than the skin nodules to account for the tuberculin reactions, and animal inoculations with emulsions of lymphatic glands from all parts of the body have proved negative. If we admit these skin lesions to be the cause of sensitisation, the main issue is whether they are caused by or contain *M. tuberculosis*, or whether some other factor, possibly a comparatively harmless acid-fast saprophyte, is the ætiological agent. If they are non-tuberculous, the fact that the development of these lesions can apparently be associated with the appearance of a sensitivity to tuberculin adds yet another difficulty to the accurate interpretation of the intradermal test; or, alternatively, the demonstration of their tuberculous nature would also depreciate to some extent the value of the tuberculin test, for with a considerable percentage of skin-lesion cases no reaction has been elicited, and most of them give some negative or very doubtful results. In an excellent summary Hagan[33] has considered the pros and cons under the seven headings we are adopting here.

### (*a*) *The Presence of Acid-fast Organisms.*

We have been able to demonstrate acid-fast organisms in eleven out of twelve cases examined. The findings of other workers vary. In our cases, we are by no means certain that it is always the same type of organism. The commonest form seen is a short, broad rod, but granular and curved forms also occur. The staining is usually

a darker red than with known tubercle organisms, but on the other hand we have seen smears which we could not differentiate from tuberculosis. Acid-fast bacilli vary to such a degree that differentiation on morphological grounds is unsatisfactory.

(*b*) *The Histological Appearance of the Lesions.*

The histological similarity between these skin lesions and true tubercles has been stressed by several workers. Admitting a considerable degree of likeness, there is no reason to assume that the formation of a granuloma with giant cells is peculiar to tuberculosis, even if acid-fast organisms are present. Runnells[35] has likened the histology to that of lupus. We hope to publish a more detailed examination of the structure of our lesions in the near future, but mention here two features that strike us as being unusual for tuberculous lesions : firstly, the very large number of plasma cells present, and secondly, the vascularity of the lesions.

(*c*) *Tuberculin Reaction.*

While a skin-lesion case will often give a reaction to tuberculin, there seems to be a consensus of opinion in American literature that the reaction is neither so marked nor so definite as in tuberculosis. Our cases were originally obtained because they gave a doubtful or inconsistent reaction, such as a small and only slight oedematous swelling in the neck, while the caudal fold test was usually negative. A variety of reactions, negative, positive or doubtful, may be obtained on retest with bovine and avian tuberculins and johnin. American evidence[30] points to a number failing to react at all, and we have met with instances where reacting animals were negative at subsequent tests. It would appear that there is a group sensitisation between Johne's bacillus and the avian tubercle bacillus and it seems possible that this might include other acid-fast organisms. Hastings, Beach and Thompson[36] obtained a transient sensitisation to intradermal tuberculin by injecting acid-fast saprophytes into tubercle-free cattle, although no visible lesions occurred.

(*d*) *Animal Inoculation.*

Many attempts have been made to infect experimental animals with skin-lesion material, but nearly all, if not all, have failed. In our own experiments guinea-pigs, rabbits, fowls, mice and calves have been used, and a variety of routes tried without success. In the few cases where acid-fast organisms are reported to have been isolated, it seems doubtful whether they were those seen in the skin lesions. Cooper and Petroff[37] found acid-fast organisms in the lymph nodes of 33 per cent. of apparently normal guinea-pigs. The suggestion that the organisms may be tubercle bacilli of attenuated virulence due to a long sojourn in the skin, as occurs in lupus, seems of doubtful admissibility. Lupus does infect experimental animals and soon regains its virulence with passage and is furthermore cultivable.

(*e*) *Culture.*

We have tried inoculating the following media, either by directly sowing fresh material or after treatment with antiformin or 6 per cent. sulphuric acid: Dorset's egg, glycerine egg, agar, serum agar, glycerine agar, glucose agar, blood agar, potato, glycerine potato, broth, cooked meat medium, Twort's medium for Johne's bacillus, Petroff's medium and Löwenstein-Jensen medium. In addition, we are at present trying a medium prepared from healthy bovine skin. Cultivation has been carried out aerobically, anaerobically, and in 10 per cent. carbon dioxide, and sometimes persisted with for six months. No acid-fast organisms have been obtained, although many other types grew, some of which were definitely present in the lesions, and not contaminants. Among other investigators Daines and Austin[19,20] claim to have cultivated acid-fast organisms, but following their technique we have met with no success. The situation is comparable to that of leprosy, where several organisms have been obtained but it is by no means certain that the causative organism has yet been isolated in pure culture[38].

(*f*) *Other Lesions in Skin-lesion Cases.*

We have found no evidence of tuberculosis in any of the twelve beasts examined. While we admit the difficulties, stressed by Schroeder[32], of making an absolutely thorough examination, we have performed careful post-mortems, which have included the removal and examination of all the major and many of the minor lymph glands, and a search of the central nervous system, as well as a thorough inspection of the viscera, and we cannot agree with him that many cases may be true tuberculosis, with lesions at an early stage or in unexpected sites. Of the large number of cases examined in America, very few have shown lesions of tuberculosis in addition to the skin nodules. In view of the fact that the technique of inoculating lymph-gland emulsions has at this laboratory detected avian and bovine tuberculosis in animals in which no macroscopic lesions were found, we feel that we should have met with some success by this method if ordinary tuberculosis had been present.

(*g*) *The Incidence of Skin-lesions in supposedly Tubercle-free Herds.*

Data on the incidence of "skin tuberculosis" in this country are very scanty. The majority of our cases have come from herds which we are reasonably certain have been free of tuberculosis for some time. In the United States the progress of eradication of tuberculosis has been marked by an increase in the number of "skin-lesion reactors" detected. Hagan[33] states "many herds may not have shown any cases of actual tuberculosis for a dozen years, and yet the skin lesions continue to appear. It is not usual for a large number of animals to show the lesions at any one time; usually there are only one or two cases at a time. Certain premises produce cases year after year, while other in the neighbourhood are not troubled

with them." Daines and Austin[21] state that of 84,620 cattle tested in Utah in 1930, 91 per cent. of the 622 reactors showed skin, subcutaneous or " no-visible " lesions, while only 51 of the 622 showed evidence of tuberculosis.

The bovine tubercle bacillus can undoubtedly produce skin and subcutaneous lesions. The Report of the Royal Commission for the Study of Tuberculosis[44] mentions many such cases, and Carpenter and Goldberg[10] produced one case. But the disease was progressive and it was always possible to infect guinea-pigs with the organisms from the lesions, even after many months sojourn in the subcutaneous tissues.

Some workers have suggested that the skin-lesions might be caused by the avian tubercle bacillus. Some skin cases certainly give a more marked reaction with avian tuberculin than with bovine, but we have found this in only about one-third of the cases we have examined. Elder and Lee[40] injected calves subcutaneously with cultures of avian tubercle bacilli and claimed to have produced a sensitisation to the intradermal tuberculin test but not to the subcutaneous. Attempts by Crawford[14], however, to infect the skin and subcutaneous tissues of cattle with avian bacilli were unsuccessful and no reaction was obtained from tuberculin tests four months later. Day[8] failed to infect fowls with skin-lesion material and similar attempts by numerous other workers, using a variety of routes, have also been uniformly unsuccessful.

It has been suggested that the subcutaneous test might be useful in differentiating between skin tuberculosis and true tuberculosis. Little exact information is available on this point though a few papers record cases which reacted to the intradermal test but were negative to the subcutaneous[3, 12, 20].

In two of our cases the animals were kept in isolation for a month, and when retested by the subcutaneous method they gave a positive reaction (see Table II). It would seem, therefore, that the sub-

Table II.

Subcutaneous Tests with Dilute Bovine Tuberculin.

| *Animal Number.* | *Average Pre-inoculation Temperature.* | *Temperature—°Fahrenheit.* | | | | | | | *Result.* |
|---|---|---|---|---|---|---|---|---|---|
| | | *Initial.* | *9th hour.* | *12th hour.* | *15th hour.* | *18th hour.* | *21st hour.* | *24th hour.* | |
| 816 | 101·6 | 101·8 | 104 | 104 | 104 | 104·4 | 103 | 103·6 | Positive |
| 817 | 102 | 102·8 | 104·8 | 105 | 104 | 103 | 102·8 | 102·8 | Positive |

cutaneous test, like the intradermal avian test, cannot be relied on to distinguish skin-lesion cases from those which are definitely tuberculous.

The possibility of the organisms being *M. paratuberculosis* must be borne in mind. So far we have been unable to cultivate them on

the special media for Johne's bacillus. Also, so far as we are aware, the incidence of Johne's disease is negligible in the herds from which our animals came.

Saprophytic acid-fast bacilli are almost ubiquitous. They can be found in butter, milk, smegma, water, dust, grass, manure and faeces. Frey and Hagan[42], using a special technique devised by Söhngen, were able to demonstrate the presence of acid-fast bacteria in a hundred samples of soil collected from various parts of the United States. There is thus ample opportunity for such organisms to gain access to the skin surface of cattle and invade open wounds, punctures, etc. The localisation of the lesions suggests the possibility of their being the result of wounds. On the other hand Crawford[14] inoculated the skin of cattle with three strains of saprophytic acid-fast organisms by scarification, intradermal injection and subcutaneous injection, but failed to produce lesions, either in or under the skin, and the animals showed no sensitisation to tuberculin up to the time of *post-mortem* examination a year later.

There is no conclusive evidence that saprophytic acid-fast organisms by themselves can cause lesions of any sort, nor that they can produce a histological picture similar to tuberculosis. The hypothesis that they may do so, however, and that they may cause a group sensitisation of an indefinite and transient sort, is one which is attracting the attention of many workers who are studying the problem.

Even if one does regard these reactions as evidence of a group sensitisation, this is no serious condemnation of the tuberculin test. According to Hastings[36] in Wisconsin, with a cattle population of $2\frac{1}{2}$ million animals, the incidence of reactors was reduced in four years from 46 per cent. to less than 0·5 per cent. Hagan[34] states that as the incidence of tuberculosis has been reduced and the number of reactors has fallen, the percentage of reactors showing skin-lesions only or no-visible lesions, has increased, although the number of such cases seems to have remained fairly constant. This is as would be expected if the sensitisation to tuberculin in these cases were due to causes other than the tubercle bacillus.

This condition is, of course, of no clinical importance, but it is of importance in relation to the tuberculin test and to the eradication of tuberculosis. In America it has a definitely patchy incidence, and in some districts it presents a serious problem to the eradication campaign. So much so that lately several writers have stressed the necessity of using the herd history to supplement the tuberculin test. Hastings[43] tested the safety of such a procedure in a number of " tubercle-free " herds, in which occasional skin-lesion cases were occurring. He concluded that such a procedure was quite feasible, and would avoid the removal of a considerable number of animals without endangering the eradication work in any way. Similar findings are recorded by Marsh[12].

It is impossible to assess the relative importance of the condition

in this country, as no data are yet available as to the incidence of skin-lesion cases in the country as a whole.

## Summary.

1. This is a preliminary report on a number of bovine skin-lesion tuberculin-reactors examined at this Laboratory.

2. The results of our examinations and a review of the literature lead us to the following tentative conclusions :—

(*a*) That the skin lesions are the most probable cause of the sensitisation to tuberculin.

(*b*) That the sensitisation is not consistently greater for avian than for bovine tuberculins, or *vice-versa* ; neither does the subcutaneous test appear to afford a means of differentiating the " skin-lesions " from true tuberculosis.

(*c*) We admit the possibility of the lesions being due to an atypical form of *M. tuberculosis*. However, in view of the microscopical appearance of the organisms, certain histological features of the lesions, failure to infect laboratory animals and failure to cultivate the organism, we are of the opinion that the acid-fast organisms seen probably do not belong to the recognised pathogenic types, although they may be associated in a group sensitisation. The acid-fast organisms may not necessarily be the primary cause of the lesion, but be secondary invaders of a wound or abscess.

3. We intend publishing further results and a detailed histological description in a future article.

## References.

[1]Perard and Ramon. (1913). *Soc. de Biol. Comptes Rendus.*, **74**, 133.

[2]Traum, J. (1916). Case reports of Lymphangitis in Cattle caused by an acid-alcoholic fast organism. *J.A.V.M.A.*, **49**, 254-257.

[3]——. (1919). Further reports on Lymphangitis in Cattle caused by acid-alcohol fast organisms. *Ibid.*, **55**, 639-652.

[4]——. (1923). Lymphangitis in Cattle caused by acid-fast organisms. *Cornell Vet.*, **13**, 240-245.

[5]——. (1928). The relation of acid-fast skin Infections of Cattle to Bovine Tuberculosis and other acid-fast infections. *J.A.V.M.A.*, **74**, 553-576.

[6]Wright, W. D. (1921). Tuberculosis Cutis. *Ibid.*, **59**, 589-602.

[7]Day, L. E. (1921). Post-mortem report on Tuberculin-Reacting Cattle. *Ibid.*, **59**, 769-773.

[8]——. (1922). Rept. Proc. 26th Ann. Mtg. U.S. Live Stock San. Assn., 1922, 46-48.

[9]——. (1928). Skin lesions in Tuberculin-Reacting Cattle. *Ibid.*, **72**, 782-787.

[10]Carpenter, C. M., and Goldberg, S. A. (1925). A study of Skin Tuberculosis. *Cornell Vet.*, **15**, 148-155.

[11]Marsh, H. (1925). Lesions in Cows reacting to the Tuberculin Test. *J.A.V.M.A.*, **68**, 185-200.

[12]Marsh, H., Warren, D. M., and Morrow, A. C. (1932). A Study of the Tuberculin Sensitization in Cattle showing subcutaneous lesions. *Ibid.*, **81**, 105-117.
[13]Crawford, A. B. (1926). Tuberculin Sensitization in Guinea-pigs caused by various Acid-fast Organisms. *Ibid.*, **69**, 579-588.
[14]——. (1928). Studies in so-called " Skin Lesion " Tuberculosis. *Ibid.*, **73**, 216-222.
[15]Mitchell, C. A. (1928). Bovine Subcutaneous Tuberculosis. *Ibid.*, **73**, 493-506.
[16]Graham, R., Thorp, F., and James, W. A. (1930). A note on Subcutaneous Tuberculous Lesions. *Ibid.*, **77**, 97-103.
[17]Feldman, W. H. (1931). A Study of the Subcutaneous Lesions of Cattle reacting to Tuberculin. *Cornell Vet.*, **21**, 268-282.
[18]Van Es, L. (1931). Remarks on Avian Tuberculosis Infection in Mammals. *J.A.V.M.A.*, **78**, 371-378.
[19]Daines, L. L., and Austin, H. (1931). Proc. 35th Ann. Mtg. U.S. Live Stock San. Assn., 1931, 414.
[20]——. (1932). A Study of so-called Skin-Lesion and No-Visible-Lesion Tuberculin-Reacting Cattle. *J.A.V.M.A.*, **80**, 414-436.
[21]——. (1932). A Study of so-called Skin-Lesions of Tuberculin Reacting Cattle. *Amer. Rev. Tuber.*, **27**, 600-610.
[22]——. (1934). Acid-fast and Non-acid-fast Micro-organisms in Skin Nodules of Tuberculin-reacting Cattle. *Ibid.*, **30**, 209-213.
[23]Report of the Chief of the Bureau of Animal Industry, U.S.A., 1932.
[24]*Ibid.*, 1933.
[25]*Ibid.*, 1934.
[26]Feldman, W. H. (1934). Sensitivity of Chickens to Tuberculin following exposure to Different Varieties of Acid-fast Bacilli. *J.A.V.M.A.*, **83**, 344-357.
[27]——. (1936). Observations on certain unidentified Acid-fast Bacteria obtained from Cattle. *Ibid.*, **88**, 166-172.
[28]Feldman, W. H., and Schlotthauer, C. F. (1935). The Isolation of the Organism of Avian Tuberculosis from Cattle. *Ibid.*, **86**, 635-643.
[29]Prendergast, W. M. (1936). *Ibid.*, **88**, 74-75.
[30]Crawford, A. B. (1936). Tuberculin reactions in Cattle showing no visible tuberculous lesions on post-mortem. *Ibid.*, **89**, 562-583.
[31]Ministry of Agriculture and Fisheries. Unpublished Reports.
[32]Schroeder, E. C. (1924). No-lesion tuberculin-reacting cattle. *J.A.V.M.A.* **64**, 544-552.
[33]Hagan, W. A. (1929). Subcutaneous Lesions which sometimes induce Tuberculin Hypersensitiveness in Cattle. *Cornell Vet.* **19**, 173-181.
[34]——. (1931). The No-lesion Case problem in the Tuberculosis Eradication Campaign. *Ibid.* **21**, 163-176.
[35]Runnels, R. A. (1932). The Histopathology of Cutaneous and Subcutaneous Nodules of Cattle. *J.A.V.M.A.*, **81**, 173-183.
[36]Hastings, E. G., Beach, B. A., and Thompson, I. (1930). The Sensitization of Cattle to Tuberculin by other than Tubercle Bacilli. *Amer. Rev. Tuber.*, **22**, 218-225.
[37]Cooper, F. B., and Petroff, S. A. (1928). " Filterable Forms " of the Tubercle Bacillus. *J. Infect. Dis.*, **43**, 200-214.
[38]Topley, W. W. C., and Wilson, G. S. (1936). The Principles of Bacteriology and Immunity. 2nd Edit. 316.
[39]Hastings, E. G. (1914). Bull. 243, Wis. Agr. Exp. Sta.

[40]Hastings, E. G., Beach, B. A., and Weber, C. W. (1924). No-lesion and Skin-lesion Tuberculin-reacting cattle. *J.A.V.M.A.*, **66**, 36-42.
[41]Elder, C., and Lee, A. M. (1924). Injection of Cattle with *B. tuberculosis* (Avian) and the Results of subsequent Tuberculin Tests. *Ibid.*, **64**, 440-450.
[42]Frey, C. A., and Hagan, W. A. (1931). The Distribution of Acid-fast Bacteria in Soils. *J. Infect. Dis.*, **49**, 497-506.
[43]Hastings, E. G., Wisnicky, W., Beach, B. A., and McCarter, J. (1933). A Detailed Study of No-lesion Tuberculin-reacting Cattle. *J.A.V.M.A.*, 82, 565-583.
[44]Royal Commission on Tuberculosis. (1911). Final Report.

## REVIEWS.

**Text Book of Pathology.** By Sir Robert Muir, Professor of Pathology, University of Glasgow. Fourth Edition. 1936. London: Edward Arnold & Co. Price 35s. net.

The appearance of a new edition of this well-known work is evidence that it is admirably adapted to the requirements of medical students and practitioners. Only minor alterations have been made in the text but apparently all important additions to knowledge in the realm of pathology have been incorporated and a few new illustrations have been added.

It remains one of the best text books on the subject. The author's great experience, not only as a teacher but also as a hospital pathologist, has given him an advantage in deciding what is appropriate and what should not be included in a work mainly intended for medical students preparing for the qualifying examination in pathology.

Very few references to matters of comparative pathology are found in the work—which is a merit and not a defect, but unfortunately one of them is not accurate. In the part dealing with paroxysmal hæmoglobinuria it is said that it " is probably allied to the paroxysmal hæmoglobinuria which is not uncommon in horses when exercised after a long period of rest," but in fact in the equine disease the period of rest that precedes an attack is nearly always a matter of a few days only.

**Experimental and Clinical Studies of the Spine of the Dog.** By Geoffrey B. Brooke, D.SC., F.R.C.V.S. Pp. 122, with 24 plates (50 figures). London: Baillière, Tindall & Cox. Price 5s.

The book has been designed to help, first, the clinician engaged in the diagnosis and treatment of diseases of the dog and, second, the comparative worker utilising observations on the lower animals in the study and elucidation of disease processes in man.

Part I is concerned with puncture of the cisterna magna in the dog, which is the routine method of reaching the subarachnoid space for the withdrawal of the cerebrospinal fluid for study. The steps in this operation are minutely described and illustrated and the characters of the normal cerebrospinal fluid in the dog are given, based on observations made by the author. This will be useful, for cistern puncture is employed as a means of obtaining samples of cerebrospinal fluid for the diagnosis or investigation of disease associated with the central nervous system, and it can also be used for bringing sera or medicinal liquids into the subarachnoid space.

Part II deals with the experimental and clinical investigations into the use of iodised oil as a radiopaque medium in the examination of the spinal subarachnoid space of the dog.

It is a piece of original work very well done.

**Variations and Diseases of the Teeth of Animals.** By Sir Frank Colyer, K.B.E., LL.D., F.R.C.S. London: John Bale, Sons and Danielsson, Ltd. 1936. Price £2 2s. 0d.

This work extends to 750 Imp. octavo pages and has 1,007 illustrations. The first part deals with variations of the teeth in number and shape in the different Orders, beginning with the Primates.

The second part relates to variations in the position of the teeth in the different Orders and extends to about 300 pages.

The remainder has separate chapters dealing with abnormal eruption of

teeth, injuries of the jaw, injuries of the teeth, overgrowth of teeth, hypoplasia of the teeth, caries, loss of tooth tissue from causes other than caries, dento-alveolar abscess, progressive destruction of the teeth sockets, and odontomes.

These figures may serve to give some idea of the immense labour which must have been devoted by the author to assembling the mass of information which the book contains regarding the abnormalities and diseases of the teeth throughout the mammalia.

It contains matter of interest to dentists, doctors, veterinary surgeons and students of zoology. Its value is greatly increased by the number and quality of the illustrations. It is impossible to withhold the hope that it will bring to the author the honour and profit which he deserves. The publishers' share in the production of the work also deserves praise.

# ABSTRACTS.

## STATISTICS REGARDING TUBERCULOSIS.

### Methods of Contaagion and Infection, and Measures for the Eradication of the Disease*

By Dr. H. Rautmann.

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In 1935 the veterinary surgeons of the Province of Saxe-Anhalt examined 35,320 herds, comprising 254,316 cattle. These herds were included in an organisation created with a view to the eradication of tuberculosis, and 12,608 of the animals belonging to 1,766 herds were examined twice during that period. The relative proportions between tuberculosis of the lungs, udder, uterus, and intestine found in these examinations were 90·9, 6·3, 2·7, and 0·08 respectively.

The microscopic diagnosis was positive only on a second examination in 9·4 per cent. of the samples of expectorate, 6 per cent. of the samples of milk, and 0·75 per cent. of the specimens from the uterus. It thus appears that the search for tubercle bacilli is especially demonstrative in discharge from the uterus.

Diagnosis was confirmed only by the inoculation of guinea-pigs in 24 per cent. of the specimens of expectorate, 20 per cent. of the milk samples, and 13 per cent. of uterine discharge. This shows that in the bacteriological examination it is the uterine exudate which gives the best results, the discharge being very rich in bacilli and consequently very dangerous from the point of view of infection.

With regard to the question of knowing in which of the clinical forms of tuberculosis microscopic examination and inoculation of laboratory animals give the greatest percentage of positive results, the following results were obtained :—12,315 samples of expectorate gave 4,335 positive results (35·20 per 100) ; 3,242 samples of milk gave 302 positive results (9·32 per 100) ; 359 samples of uterine mucositis gave 133 positive results (37·05 per 100) ; 59 samples of fæces gave two positive results (3·39 per 100).

In practice the suspicion of uterine tuberculosis is thus more frequently confirmed in the laboratory than suspected pulmonary or mammary tuberculosis, and still more frequently than intestinal tuberculosis.

In 1935 there were taken for examination from the herds brought under the Society for the Eradication of Tuberculosis 15,975 samples, with 4,772 positive results. From herds not inscribed under the Society 327 samples were examined, so that the total number of bacteriological examinations was 16,302 with 4,858 positive results. The samples of milk examined came from 57,842 herds, and gave 344 positive results.

In the examination of milk it was observed that in cases in which the result was positive one had not in every instance to deal with a tuberculous mastitis, samples being sometimes contaminated with tubercle bacilli as a result of lack of cleanliness.

The percentage of infection in herds is directly proportional to their size. Clinical examination shows that while the average number infected in the whole of the herds is 11·53 per 100, it is only 7·82 per 100 in the herds with from 1 to 10 animals, 26 per 100 in the herds with 11 to 25 animals, 52·77 per 100 in herds with 26 to 50 animals, and finally 70·74 per 100 in herds with more than 50 animals.

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* From *Deut. tierärzt. Wochenschrift*, Sept., 1936.

The result of the clinical examination also depends on the employment of tuberculin.

The statistics prove that tuberculosis cannot really be held in check when one has some small stables containing only a few animals. They also prove that the respiratory route constitutes the principal entrance of tubercle bacilli, and that in a large herd a single animal with tuberculous cavities in the lungs rapidly infects the majority of the animals co-habiting with it.

The compensation paid by the State has been raised on an average to 974·04 gold marks for each animal slaughtered under the regulations; the deduction made for the value of the flesh and skin was of the mean value of 159·77 gold marks; the mean compensation paid was raised to 113·27 gold marks for each animal slaughtered officially; the deduction made from the value of the flesh and skin was of a mean value of 159·77 gold marks; the average compensation allowed was raised to 113·27 gold marks. Of the 5,947 animals slaughtered officially in 1935, 4,834 (81·2 per cent.) were sold to the butcher, 696 (11·7 per cent.) as second-class meat, and 417 (7·02 per cent.) to the knacker.

If on examination of an animal slaughtered officially the experts consider that the proprietor has acted in an improper manner, for example, in making a declaration too late, the compensation allowable is diminished by from 10 to 20 per cent. If the owner has wilfully failed to carry out the recommendations made with a view to the eradication of the disease he does not receive compensation from the State and is given only the sum realised by the sale of the flesh and the hide.

Total compensation by the State is payable only during the first four years of the owner's membership of the organisation against tuberculosis. After this delay the sum payable is reduced by 50 per cent. During the first four years the State provides the tuberculin free, the cost of the purchase being compensated by the reduction of the compensation given to negligent owners.

In 79 cases of suspected pulmonary tuberculosis the positive diagnosis made in the laboratory was not confirmed at the autopsy. These 79 cases comprised 52 that were positive by microscopic examination and 22 cases positive to experimental inoculation of laboratory animals. These errors are not due to the employment of improper instruments or to the fact that the subjects were carriers of bacilli at the moment when the samples were taken; they are due to the presence of tubercle on the respiratory mucous membrane which the microscope puts in evidence before they have had time to provoke macroscopic lesions in the lungs.

A study of the different routes by which tubercle bacilli penetrate proves that the rearing of calves and young cattle in a separate stable is an absolute necessity. The bacilli penetrate into the body by the respiratory, digestive and genital passages. It is certain that tuberculosis may be congenital, and in the calf appreciable macroscopic lesions in the first weeks of life are due entirely to intra-uterine infection. In fact at that date after birth the bacilli which may be introduced either by the respiratory passages or by the alimentary way have not had the time necessary to provoke visible macroscopic lesions.

In the adolescent animals tuberculosis of alimentary origin is much less frequent than tuberculosis of respiratory origin. This infection is realised by droplets or by tuberculous dust. But the infection by droplets is realised directly by inhalation, while the dust from desiccated, finely pulverised expectorate rises in the atmosphere. The tuberculous droplets are infective only in the immediate vicinity of the sower of bacilli, while infection by dust may operate throughout the whole stable.

An example is reported showing that the infection by tuberculous dust is very dangerous for young children. Of 100 school children of the age of 8 years in a particular village, 90 gave a positive reaction to tuberculin and clinical signs were already present in a certain number of them. The infection had been derived from a particular teacher affected with serious pulmonary tuberculosis. The Supreme Court of the Reich allowed compensation at the expense of the State to the children contaminated by this tuberculous teacher. Infection by way of the alimentary canal was impossible in this class, and, infection by virulent droplets was scarcely probable, since these fall rapidly on the ground and cannot be projected for more than a metre and a half. But when spread on the ground they become desiccated and the dust rises and is disseminated throughout the whole class by currents of air which penetrate into the finest branches of the bronchi. It is the same with tuberculous dust derived from the expectorate of cows with cavities in the lung. The tuberculous dust when inhaled penetrates into the lung, where it is retained for a certain time by the bronchial mucus. This circumstance explains the momentary presence of bacilli in animals free from any lesion and not infected.

Young cattle found in a stable in which several cows are distributing bacilli become infected to the same extent as these children.

The control of the sanitary condition of 143 herds was effected by the tuberculin test in 2,352 cattle, of which 32 bulls and 7 steers were kept in cohabitation with 1,121 cows. Researches were also carried out on 544 heifers of more than one year old and 648 younger heifers. The intracutaneous tuberculin test revealed 71·8 per cent. of reactors in the bulls, 71·4 in the steers, 71·2 in the cows, 46·3 per cent. in the heifers over one year old, and 20·9 per cent. in those under a year old. The receptivity had been practically equal for the bulls, steers and cows kept in the same stable. The percentage was notably lower in the heifers of more than a year old, of which a part only were kept in the common stables and had been exposed for a relatively short time to contagion.

If the infection by tuberculous dust plays a great role in the cow-shed, that by tuberculous droplets operates specially among the working oxen. If one of the two oxen yoked together makes some violent expirations under the stress of the work it projects its tuberculous droplets over a radius of at least one metre. The projected droplets are inhaled by its fellow worker, and after some months the latter is infected. This contagion can be effected also in the stable, but in a way less distinct, because the pair of working oxen are generally neighbours in the stable.

In the 143 herds submitted to the tuberculin test, 17, or 11·8 per cent., were free from tuberculosis. In nearly all the cases these herds were small, while in the herds comprising more than 20 animals they were all more or less infected.

In the struggle against tuberculosis regard must be paid in the first place to the young animals, which should be isolated from birth in a special stable. The statistics collected show that among animals thus isolated there are few or no positive reactions to tuberculin, while contagion is the rule in the common stables. It is therefore important that farm buildings constructed in the future should possess isolation stables for the young animals.

Of 177 tuberculous calves reported by the veterinary inspectors of meat, all with the exception of two had tuberculosis of the liver or hepatic lymphatic glands (98·2 per cent.). In 17 of these calves these were the only lesions discovered. One may therefore conclude that the infection occurred through the umbilical vein.

On the other hand, in the calf the generalisation of tuberculous lesions is very precocious. In the calves attacked with pulmonary tuberculosis lesions of the bronchial and mediastinal glands were absent only in 24 cases, which points to the infection of the organs and their glands during the fœtal period. All the tuberculous lesions observed in the calf were undergoing caseous transformation—another certain indication of ante-natal infection.

In 43 tuberculous calves tuberculous lesions were found in the intestine and the afferent lymphatic glands associated with the extra-digestive lesions. The fact that one has found 175 cases of hepatic tuberculosis, 152 cases of pulmonary tuberculosis, and only 43 cases of intestinal tuberculosis tends to show that the intestine plays only a very secondary role in the genesis of tuberculosis in the calf. Moreover, the 43 cases of tuberculosis of the intestine are far from constituting in every case a primary localisation ; on the contrary, the majority are manifestly due to a generalisation from primary foci situated in the liver or in the lungs.

In the 177 tuberculous calves, 61, or 34·5 per cent., showed tuberculosis of the spleen. The percentage in which the kidneys and the intermuscular lymphatic glands were affected was respectively 13·5 and 16·4 per cent. One knows, moreover, that these lesions, so frequent in the kidneys, spleen, and the intermuscular lymphatic glands, are very rarely observed in cattle that have contracted the disease after birth.

The discovery of 177 tuberculous calves led to the detection of 69 tuberculous mothers ; 45 showed tubercle bacilli in the uterine mucus ; in the milk the bacilli were present in 5, and in 34 the bronchial mucus also contained the bacilli. The search for tuberculous calves plays a great role in the discovery of centres of infection.

Cows affected with tuberculosis of the peritoneum are also often the subjects of tuberculous metritis. In 298 cows lesions were found in the uterus in 247, or 82·9 per cent. of the cases. Tuberculous metritis thus constitutes the most frequent epiphenomenon of tuberculous peritonitis ; it is only exceptionally that it results from generalisation by way of the blood stream.

To sum up, in the calf tuberculosis most frequently has its origin in tuberculous metritis ; it is congenital and rarely due to milk derived from a tuberculous udder. The respiratory route plays an important role from birth and contagion occurs in this way notably in the stables with one or several cows affected with open tuberculosis of the lung.

## EXPERIMENTAL STUDIES REGARDING IMMUNITY IN BANG'S DISEASE.*

By Prof. Dr. Paul Hofmann.

*From the Institute of Hygiene of the Technical High School in Dresden.*

Hitherto the prophylactic and therapeutic measures opposed to bovine contagious abortion have been in the first line directed to the production of immunity, namely, inoculation with living or dead cultures or of Bang's bacilli, or metabolic products extracted from these.

The inoculation with living organisms has in recent times suffered a strong limitation on the part of the State and is now permitted only in special cases. It has been recognised that it does not lead to success and is

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open to other serious objections. Such inoculation can neither cure the disease nor actually arrest it in an infected herd ; in the opinion of some, but not all, it can at the most prevent the most striking symptom of the disease —actual abortion. According to Zeller's investigations the difference in the number of abortions before and after inoculation with living culture is inconsiderable. (Zeller. *Münch. tierärzt. Wschr.*, 1933).

Inoculation with dead organisms or metabolic products or extracts of Brucella bacteria has the advantage over living cultures that " if it does no good it does no harm." During recent years a considerable number of such vaccines have been recommended and experiments with them have been published by different authors. Most of them are put forward as substitutes for living organisms, but apparently none of them has achieved undoubted success. In general, where results appear to have been favourable the vaccination was combined with the strict application of hygienic measures.

The unsatisfactory results of inoculation procedures raise the question whether there is actually attainable an acquired immunity against Brucellosis. It is beyond any doubt that after natural infection with Brucella bacteria, as well as after artificial administration of these organisms or their antigens, specific immune bodies, such as agglutinin, precipitin, etc., are actually formed and can be recognised in the blood serum, and partly also in the milk serum. It is doubtful, however, whether those immune bodies, which are so important for diagnosis, can be accepted as proof of an immunity that is active against the infection and can help to free the body from the causal bacteria.

The conditions with regard to the development of immunity in Brucellosis have not yet been fully explained. To them, however, belongs the decided role in the determination of the question whether the disease in our herds can be stamped out through an artificial immunisation.

The author has for a period of three years been engaged in an experimental study regarding the conditions of immunity in Brucellosis. The experimental animals used were rabbits, guinea-pigs and mice. The condition of the experimental animals in respect of health were constantly maintained, and the body temperature and weight were regularly determined. All the animals intended for experiment were first tested several times as to their freedom from immune bodies against *Br. abortus*.

The antigens used were prepared in five different ways and ten different strains of virulent *Br. abortus* were used in their preparation. The strains were cultivated from infected cows or from fœtuses. There were used for inoculation fully virulent living bacteria, a Bang vaccine (dead culture) and also a vaccine which was a mixture of albumose and Brucella organisms ; further, a vaccine composed of live broth culture of the *Br. abortus*, and a mixture of dissolved Bang-bacteria albumose and Bang-bacteria broth filtrate.

The living material used for inoculation was composed of fully virulent *Brucella abortus* which had been cultivated for 4 days at 37° C. in liver broth or in cystin broth. Such broth culture was thoroughly washed before it was inoculated into the experimental animals.

The Bang-vaccine was made from a layer of growth on cystin agar plates which had been kept for 4 days at 37° C. in a $CO_2$ atmosphere. The plates were flooded with normal saline, and the suspension was kept in a water-bath at 60° C. for 1 to 2 hours in order to kill the organisms. Each 1 c.c. of the vaccine contained about one hundred million organisms.

The albumose of the brucella organisms was obtained from cultures

which had been incubated for 48 hours at 37° C. on glycerine agar plates with a little distilled water on the surface. The culture thus obtained was washed several times with distilled water in order to remove any adherent material from the culture medium. The precipitate was left for 24 hours in the ice chest at 40° C. and was then suspended in 1,000 c.c. of 0·2 per cent. $H_2SO_4$ and kept for three weeks at 37° C.

It was then neutralised with baryta solution (indicator litmus) and left standing for 24 hours. At the end of that period it was strongly centrifuged. The cell-free supernatant liquid contained the hydrolysis products of the bacterial albumose, of which the biuret reaction was positive.

There had first to be proved whether the albumose dissolved out of the bodies of the organisms and the filtrate of the broth culture of the organisms had a toxic effect. The question whether these organisms form toxic materials has repeatedly been examined. The English Committee (Epizootic Abortion, Report of the Departmental Committee appointed by the Board of Agriculture and Fisheries, 1909) found that Bang's bacilli produced no toxin in serum-glycerine broth cultures ; germ-free broth filtrates produced no anaphylactic shock in pregnant heifers infected with contagious abortion. Reichel and Harkins extended the experiments of the English Commission and found that cultures of Bang's bacilli containing peptone formed toxin (peptotoxin), but cultures on nutrient medium free from peptone did not.

Through washing, the bacteria grown on the medium containing peptone were freed from peptotoxin and the alcoholic precipitate of the germ-free wash water contained peptotoxin, but the only animals that reacted to peptotoxin were those previously sensitised. The experiments carried out by Holth show that the bacteria-free filtrate of broth cultures of Bang's bacilli is not toxic for white mice and rats by either intraperitoneal or subcutaneous inoculation in 1 c.c. doses. Also precipitates of culture filtrates with alcohol or with sulphate of ammonia when treated with alcohol are not toxic to experimental animals.

The author's tests of the antigenic effect of his inoculation materials were carried out on rabbits and guinea-pigs.

The experimental animals were given altogether five gradually increasing doses with intervals of a week. The injections were given subcutaneously, intravenously, intraperitoneally, and by the mouth. The doses for each sort of vaccine were kept as uniform as possible. The antigen action was measured by the occurrence of agglutinin- and complement-binding amboceptors in the serum of the experimental animals. Blood was obtained from the rabbit by puncture of a vein of the ear and from guinea-pigs by puncture of the heart. The antibody content of the serum of the experimental animals was first tested six days after the first injection. Thereafter there were five further tests with intervals of a week ; six months after the first inoculation a serological examination was carried out. As test bacteria for the agglutination, suspensions of living Bang and *melitensis* cultures in normal saline were used. For the complement-fixation tests titrated extracts of Bang and *melitensis* serum glycerine broth cultures which had been incubated for eight weeks at 37° C. were used as antigens. In details the author proceeded as in researches in human Bang infections. As regards the result of the reactions it was found to be indifferent whether Bang or *melitensis* cultures or their extracts were used as test materials. In the course of the researches the author also came to the conclusion that the differentiation of the *Br. abortus* Bang and *melitensis* bacteria cannot be made by agglutination and complement-fixation reactions. The serum of the experimental

animals for the agglutination tests was used in the following proportions:—1 : 50; 1 : 100; 1 : 500; 1 : 1,000; 1 : 2,000; 1 : 10,000, diluted with normal saline. For the complement-fixation reactions the measurements were:—0·05; 0·03; 0·02; 0·01; 0·005, and 0·001 c.cm. For each substance used for inoculation and for each different method of application three rabbits and three guinea-pigs were used. Apart from unimportant individual variations all the animals in each experiment reacted in the same sense.

A Table in the article gives the average agglutination and complement-fixation titre of the sera of the rabbits and guinea-pigs inoculated with living culture, dead vaccine, bacterial protein, broth culture filtrate, and a mixture of the last two. It shows that none of these materials when administered by the mouth had any antigenic action in rabbits. In guinea-pigs, on the other hand, there was a slight antigenic effect after oral administration of living culture, vaccine, and broth culture filtrate, but not of bacterial albumose or the mixture of bacterial albumose and broth culture filtrate. On parenteral administration all the materials used had an antigenic effect, but the degree of antibody production varied with the animal species, the method of injection, and the particular material. The production of antibody was generally stronger in the guinea-pigs than in the rabbits, but otherwise the serological behaviour in the two species was almost the same. With intravenous injection the antigenic effect was almost always greater than with intraperitoneal or subcutaneous administration. Of the different agents used live culture had the greatest antigenic effect. In rabbits it exceeded the vaccine in agglutinin formation by about 30 per cent., the broth culture filtrate about 70 per cent., the bacterial albumose, and the mixture of bacterial albumose and broth culture filtrate by about 80 per cent. In guinea-pigs the effect of the different agents on the formation of agglutinin was similar. After parenteral treatment with living culture even the complement-fixation substances were formed in greater quantities in the serum of rabbits and guinea-pigs than after the administration of the other agents.

Further, the experimental animals inoculated with live culture reached absolutely the highest titre, the antibody production set in quicker, and the antibody circulating in the blood within the observation period of six months disappeared more slowly than in the case of the other vaccines.

The inoculations with the dead vaccine, the broth culture filtrate, and the bacterial albumose were well borne by all the experimental animals. In some cases the parenteral application of these preparations, most frequently after the use of the vaccine, led to induration and abscess formation at the site of injection, but the animals showed neither loss of weight nor fever. The *post-mortem* of animals killed after a period of six months' observation showed no lesions. In the animals treated with living agents there occurred without exception loss of weight and elevation of temperature, after parenteral inoculation the site of injection frequently became indurated or an abscess was formed there, and Brucella organisms were regularly found in the pus of the abscess. In the experiment in which the guinea-pigs were killed after six months, swelling of the spleen, liver and lymphatic glands was generally observed in them. The spleen and liver were soft, friable, and filled with specks and nodules; the mesenteric lymphatic glands were frequently suppurating and in some cases there was purulent softening in the medullary cavity of the ribs. Brucella organisms could frequently be cultivated from the diseased organs. Of the rabbits inoculated with living culture, when killed after six months only two animals showed organ lesions the same as guinea-pigs.

For testing the prophylactic effect of the vaccines six rabbits and guinea-pigs, and ten mice were inoculated subcutaneously with Bang living culture, Bang vaccine, Bang bacterial albumose, broth culture filtrate, and a mixture of the last two. About eight weeks after the termination of this treatment the rabbits and guinea-pigs were subcutaneously inoculated with 0·2 c.c. of a four-days-old liver broth live culture. Four control animals which had not previously been treated were similarly submitted to infection.

The experiment was terminated about six months after the infection.

The experimental animals treated with live culture showed loss of weight and elevation of temperature. In the six months following the infection these symptoms occurred in four of the six rabbits and seven of the ten mice, but only in one of the six guinea-pigs. The experimental animals previously treated with Bang vaccine, bacterial albumose and broth culture filtrate showed some weeks after the infection the same picture as the animals previously treated with live culture and the control animals. After the conclusion of the experiment the guinea-pigs, mice, and rabbits showing symptoms were killed and dissected. The usual organ changes were present and Bang organisms could be cultivated from the affected organs.

According to these experiments no prophylactic effect can be ascribed to any of these vaccines. The circumstance that only a comparatively small number of rabbits and mice became clinically ill and on section showed organ changes cannot be interpreted as the effect of prophylactic inoculation but is obviously attributable to the susceptibility of rabbits and mice for brucella bacteria.

The results of the experiments with the control animals, and in the guinea-pigs, which are specially susceptible to brucella infection, confirm that.

The testing of the therapeutic action of the different materials was carried out on guinea-pigs, 27 in number. Each animal was first inoculated subcutaneously with 0·2 c.c. of a four-days-old living *Br. abortus* liver broth culture. For the determination of the antigen effect, about four weeks after this infection, each lot of six guinea-pigs was treated in the manner already described with Bang vaccine, bacterial albumose, broth culture filtrate, and a mixture of bacterial albumose and broth culture filtrate [that is to say, the groups of guinea-pigs were treated respectively with these things]. In each experiment three animals were left untreated to serve as controls. At the end of six months the experimental animals were killed and dissected. Except in three guinea-pigs, of which one had been treated with Bang vaccine, one with Bang-bacterial albumose, and one with broth culture filtrate, there occurred an elevation of temperature and a diminution of body weight. On *post-mortem* examination there was found in these animals the well-known organ lesions in equal severity. From the diseased organs Brucella organisms could be cultivated in nearly all the cases. The result of this experiment show that the vaccines used had no therapeutic activity.

It was also shown that the Bang-bacteria albumose, in the doses in which it was used in the tests for prophylactic and therapeutic activity, appeared to exert no influence on the brucella infection, since the experimental animals treated with Bang-bacteria albumose showed in respect of the condition of infection with Bang-bacteria the same picture as the experimental animals which had been treated with Bang vaccine or broth culture filtrate.

Although the vaccines used in the experiments to some extent exhibited a strong antibody-producing effect on the experimental animals, no actual

immunity ensued. That showed that in brucella infection the formation of agglutinins and amboceptors is no proof of the presence of immunity against the infection. In dealing with Brucellosis without risk one must abstain from measures which depend for success on the production of antibodies, and rely more on the carrying out of measures of hygiene, to which the greatest value must be assigned. Even in difficult conditions it is also almost always possible thereby to limit the distribution of the infection and to check the spread of the disease.

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